

Inorganic nanolayers: structure, preparation, and biomedical applications

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Abstract: Hydrotalcite-like compounds are two-dimensional inorganic nanolayers also known as clay minerals or anionic clays or layered double hydroxides/layered hydroxy salts, and have emerged as a single type of material with numerous biomedical applications, such as drug delivery, gene delivery, cosmetics, and biosensing. Inorganic nanolayers are promising materials due to their fascinating properties, such as ease of preparation, ability to intercalate different type of anions (inorganic, organic, biomolecules, and even genes), high thermal stability, delivery of intercalated anions in a sustained manner, high biocompatibility, and easy biodegradation. Inorganic nanolayers have been the focus for researchers over the last decade, resulting in widening application horizons, especially in the field of biomedical science. These nanolayers have been widely applied in drug and gene delivery. They have also been applied in biosensing technology, and most recently in bioimaging science. The suitability of inorganic nanolayers for application in drug delivery, gene delivery, biosensing technology, and bioimaging science makes them ideal materials to be applied for theranostic purposes. In this paper, we review the structure, methods of preparation, and latest advances made by inorganic nanolayers in such biomedical applications as drug delivery, gene delivery, biosensing, and bioimaging.

Keywords: inorganic nanolayers, layered double hydroxides, layered hydroxy salts, drug delivery, biosensors, bioimaging

Introduction

In recent years, inorganic metal nanolayers have been receiving increased attention from scientists of various fields, due to their fascinating properties, such as ease of preparation, ability to intercalate variety of anions like inorganic anions, organic anions, biomolecules, and genes, and tendency to protect intercalated anions from physicochemical degradation and sustained release of intercalated anions. These inorganic nanolayers have a hydrotalcite-like structure. The most common class of these inorganic nanolayers is well known as layered double hydroxides (LDHs), and another type is referred to as layered hydroxide salts (LHS). Several books on this research field have provided valuable insight into their functionality and versatile applications. One popular book by Rives¹ has presented a great overview of inorganic nanolayers. In addition, Duan and Evans² clearly explain the inherent molecular dynamics in play, whereas Carillo and Griego et al³ discussed various applications and methods of preparation. Many reviews on LDHs, such as Del Hoyo,⁴ Khan et al⁵ (drug-delivery applications of LDHs), Wang and O'Hare⁶ (synthesis and applications of LDHs), and Rives et al^{7,8} (drug-delivery applications, drug intercalation of LDHs and sustained release), have been published.

Drug delivery, gene delivery, biosensing science, and bioimaging technology are the key tools of modern-day biomedical sciences. Different materials are used for the application of the aforementioned biomedical tools but there are certain issues related to

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Figure 1 Applications of inorganic nanolayers.

different materials, such as cytotoxicity, lack of biodegradation, and different types of material being used for individual application.⁹ Inorganic nanolayers have many advantages for biomedical applications, such as having been proven to possess very high in vitro and in vivo biocompatibility and biodegradability, and a single material can be applied for drug delivery, gene delivery, biosensing, and in bioimaging sciences. Figure 1 shows the different applications of inorganic nanolayers. This review addresses several aspects, such as structure, preparations, various biomedical sustained-release properties, in vitro and in vivo biocompatibilities, and therapeutic applications. There have been many reviews regarding inorganic nanolayers, but most of them have focused on one dimension, ie, their drug-delivery applications.

This is a comprehensive review that provides detailed information about the structure, preparation, and most recent advances in biomedical applications, such as drug delivery, gene delivery, biosensing, and bioimaging, of inorganic nanolayers.

Structure

Structure of brucite

In layered magnesium hydroxide (brucite) $[\text{Mg}(\text{OH})_6]$, the divalent M^{2+} ions are octahedrally surrounded by hydroxide ions. These octahedral units of magnesium hydroxides share their edges and form infinite layers in which O–H

forms the bond perpendicularly to the layers. In these layers, hydroxyl anions are arranged in a closed-packed manner with triangular symmetry in two-dimensional (2-D) planes. The octahedral holes of the O–H alternate planes are occupied by Mg^{2+} ions, accounting for the triangular lattice similar to the one occupied by O–H ions, resulting in neutral layer.^{10–14} The structure of brucite is shown in Figure 2.

Structure of layered double hydroxides

Compositional changes can be made in the brucite structure by the isomorphic replacement of divalent cations with trivalent cations (M^{3+}). This results in overall positive charge in the layer and must be counterbalanced by negatively charged anions. In nature, hydrotalcite is a mineral with a similar isomorphic structure. It consists of hydroxycarbonate of magnesium and aluminum, which can be easily crushed to powder. The foliated structure has the formula $\text{Mg}_6\text{Al}_2(\text{OH})_{16}\text{CO}_3\cdot 4\text{H}_2\text{O}$.^{15,16}

Substitution of divalent magnesium cations by the trivalent Al^{3+} results in overall positive charge in the hydrotalcite.¹⁵ LDHs are often described as hydrotalcite-like compounds, as both hydrotalcite-like compounds and LDHs have isomorphic substitution of the trivalent cations, with a structure similar to brucite, such as layers with positive charge and charge-neutralizing anions and solvent molecules

Table 1 Biomedical application of layered double hydroxides (LDHs)

Sample	Type of formulation	Name of formulation	Type of disease	Reference(s)
1	Anticancer formulation	Methotrexate LDHs	Bone cancer, leukemia, etc	Zhang et al, ¹¹⁴ Oh et al ¹¹³
2		Ifosfamide LDHs	Pediatric, adult tumors, soft-tissue sarcomas and lymphomas	Nie and Hou ¹¹⁷
3		Camptothecin LDHs	Lung cancer, ovarian cancer, pancreatic cancer, stomach cancer	Wu et al ¹²⁰
4		Protocatechuic acid LDHs	Cervix, breast, human leukemia (pa-2000-leukemia), liver, lungs, prostate	Barahuie et al ¹²²
5		Etoposide-Mg/Al LDHs	Small-cell lung carcinoma, gastric cancer cells, hematologic malignancies, childhood malignancies, germ-cell tumors	Qin et al ¹²⁸
6		Ciprofloxacin LDH	Adenocarcinomic human alveolar basal epithelial cancer cell line to demonstrate synergistic effect	Latip et al ¹²⁹
7	Antimicrobial formulation	Ciprofloxacin LDH	<i>Pseudomonas aeruginosa</i>	Hesse et al ¹³⁴
8		Hippuric acid ZnLH	<i>P. aeruginosa</i> , <i>Staphylococcus aureus</i>	Hussein et al ¹³⁶
9		Benzylpenicillin-Mg/Al LDHs	<i>Micrococcus lysodeikticus</i> and sulfate-reducing bacteria (SRB)	Wang et al ¹⁶
10	Antimicrobial biomaterial	Amino acid-Mg/Al LDHs	<i>P. aeruginosa</i>	Valarezo et al ¹³⁹
11		Ag-Zn/Al LDHs	<i>Escherichia coli</i> , <i>S. aureus</i>	Mishra et al ¹⁴²
12		AgNP _{bio} -Mg/Al LDHs	<i>E. coli</i> , <i>S. aureus</i>	Marcato et al ¹⁴⁶
13		AgNP-Zn/Al LDHs	<i>S. aureus</i> (ATCC 25923), <i>E. coli</i> (ATCC 35218)	Carja et al ¹⁴⁷
14		Zn-Ti LDHs	<i>Saccharomyces cerevisiae</i> , <i>S. aureus</i> , <i>E. coli</i>	Zhao et al ¹⁴¹
15		ZnNP-Zn/Al LDHs	<i>E. coli</i> , <i>S. aureus</i>	Zhang et al ¹⁴⁸
16	Antituberculosis formulation	PAS ZnLH	<i>Mycobacterium tuberculosis</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>Candida albicans</i>	Saifullah et al ^{137,152}
17	Antituberculosis formulation	PAS-Zn/Al LDHs	<i>M. tuberculosis</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>C. albicans</i>	Saifullah et al ^{123,153}
18		Isoniazid-Zn/Al LDHs	<i>M. tuberculosis</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>C. albicans</i>	Saifullah et al ¹⁵⁵
19		Isoniazid-Mg/Al LDHs	<i>M. tuberculosis</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>C. albicans</i>	Saifullah et al ¹⁵⁴
20	Sunscreen formulation	Cinnamic acid ZnLHs	Protection from UV radiation	Mohsin et al ¹⁴²
21		Cinnamic acid-Zn/Al LDHs	Protection from UV radiation	Mohsin et al ¹⁰¹
22		Benzophenone-4-Zn/Al LDHs	Protection from UV radiation	Mohsin et al ¹⁰¹
23		Eusolex® 232-Zn/Al LDHs	Protection from UV radiation	Mohsin et al ¹⁰¹
24		Benzophenone-9-Zn/Al LDHs	Protection from UV radiation	Mohsin et al ¹⁰¹
25	UV-protective coating material	Fe ³⁺ with Mg/Al LDH polysiloxanes	Protection from UV radiation	Wang et al ¹⁰⁰
26		2,4-dihydroxybenzophenone dodecylbenzenesulfonate LDHs	Protection from UV radiation	Li et al ¹⁰³
27	Gene-delivery formulation	pEGFP-N1 DNA LDHs	Transfected into mouse motor neuron cells (NSC 34)	Li et al ¹⁶²
28		Plasmid DNA LDHs	COS7 cells and HepG2 cells	Hu et al ¹⁶³
29	Gene-delivery formulation	DNA LDHs	DNA protection in adverse environment of Cd ²⁺ /Pb ²⁺ solution	Wu et al ¹⁶⁴
30	Gene-delivery and anticancer formulation	siRNA LDHs	Enhanced siRNA stability	Zhang et al ¹⁶⁶
31		siRNA-5-FU LDHs	Cancer treatment	Li et al ¹⁷³
35		siRNA LDHs	Transfected into cytoplasm of HEK293T cells	Ladewig et al ¹⁶¹

Abbreviations: ZnLH, zinc layered hydroxides; NP, nanoparticle; PAS, *para*-aminosalicylic acid; UV, ultraviolet; DNA, deoxyribonucleic acid; siRNA, small interfering ribonucleic acid; 5-FU, fluorouracil.

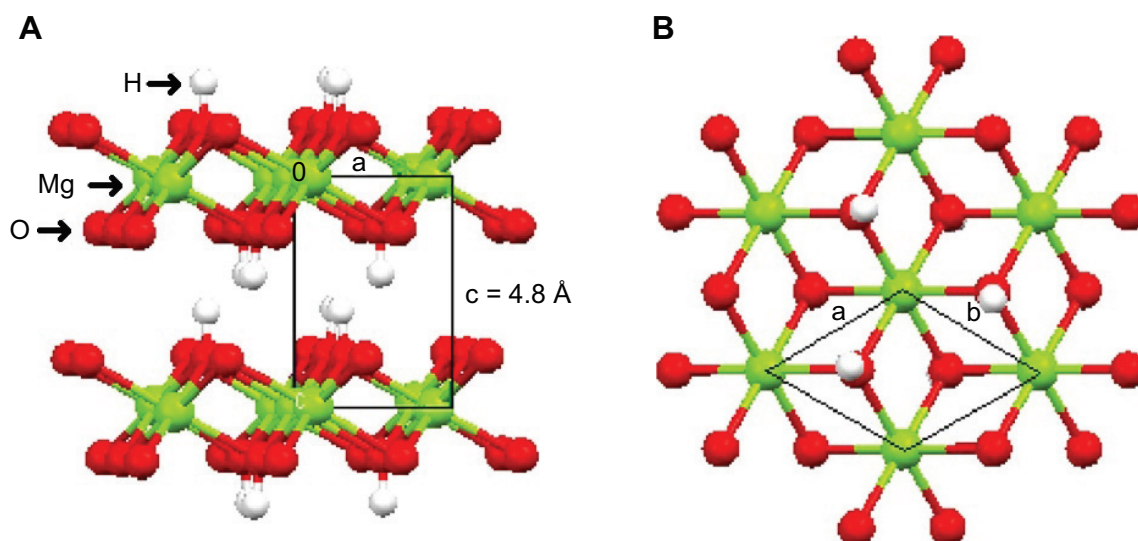


Figure 2 Schematic representation of the brucite structure.

Notes: (A) Side and (B) top view of the layer. Reprinted from Arizaga GG, Satyanarayana KG, Wypych F. Layered hydroxide salts: synthesis, properties and potential applications. *Solid State Ionics*. 2007;178:1143–1162, Copyright 2007, with permission from Elsevier.¹¹

within the interlayer galleries.^{2,7,8,17,18} The general formula for LDHs is $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{x+}(A^{m-})_{x/m} \cdot nH_2O$, where M^{2+} are divalent cations, such as Mg^{2+} , Zn^{2+} , Ni^{2+} , and Ca^{2+} , M^{3+} are trivalent cations, ie, Al^{3+} , Fe^{3+} , and Cr^{3+} , and A is a counter anion with negative charge (m).^{2,8,10,12,18} They have an octahedral structure, in which metal cations are accommodated in the centers of the edge-sharing octahedral; each cation contains six OH^- ions that are pointed toward the corners and form infinite 2-D sheets.^{1,2,7,8} Figure 3 shows the schematic structure of LDHs with side view and top view.

Structure of layered hydroxides

One way of modification of brucite-like structures is by induction of trivalent cations similar to LDHs. Another way

of structure modification is by replacing the hydroxyl group (OH^-) with suitable anions or water molecules. These types of compounds are called layered hydroxides or LHS, with the general formula $M^{2+}(OH^-)_2 \times (A^{m-})_{x/m} \cdot nH_2O$, where M^{2+} is the metal cation (eg, Mg^{2+} , Zn^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} , and Cu^{2+}) and A^{m-} is the counterion. In the layered hydroxide structure, additional anions must be located in the second coordination sphere.^{2,11,18} Figure 4 shows the structure of LHS with both the side view (Figure 4A) and top view (Figure 4B).

Preparation methods of layered double hydroxides

LDHs can easily be prepared by a number of methods, such as:

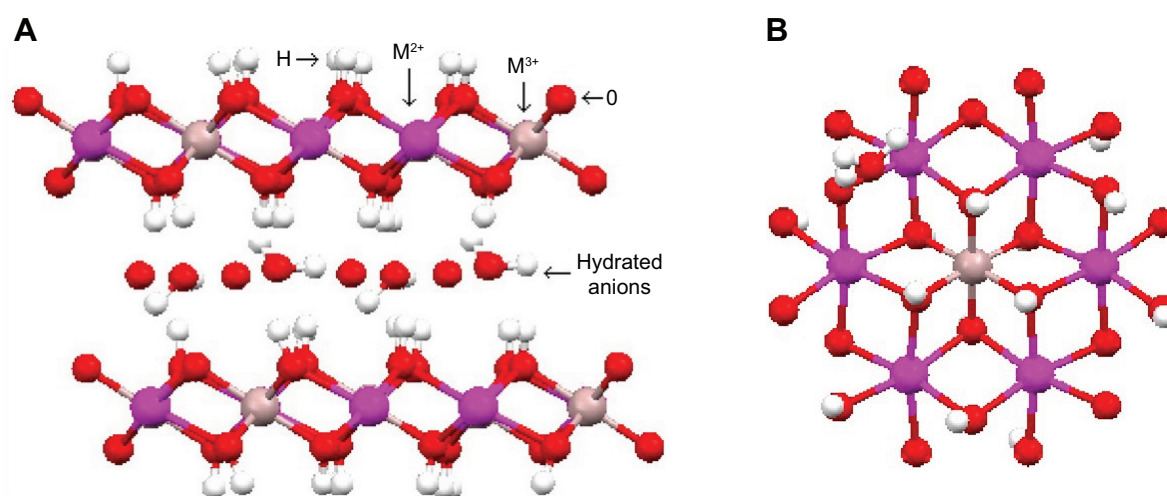


Figure 3 Schematic representation of the structure of layered double hydroxide.

Notes: (A) Side and (B) top view of the layer. Reprinted from Arizaga GG, Satyanarayana KG, Wypych F. Layered hydroxide salts: synthesis, properties and potential applications. *Solid State Ionics*. 2007;178:1143–1162, Copyright 2007, with permission from Elsevier.¹¹

Table 2 In vitro biocompatibility studies of layered double hydroxides (LDHs) toward various cell lines

Sample	Type of LDH	Biocompatibility with cell type	Reference
1	Etoposide (VPI 6)–Mg/Al LDHs	Human GES-I cells	Qin et al ¹²⁸
2	Perindopril erbumine LDHs	Human liver cell line	Hussein et al ¹²⁴
3	pEF-eGFP LDHs	HEK 293T cells	Xu et al ¹⁷⁴
4	Protein gene LDHs	Monkey kidney (Vero3) cells	Masarudin et al ¹⁸³
5	Salicylic acid ZnLHs	Monkey kidney (Vero3) cells	Ramli et al ¹⁸⁴
6	LDH-AgNP _{bio}	Permanent lung fibroblast cell line (V79)	Marcato et al ¹⁴⁶
7	<i>Para</i> -aminosalicylic acid–Zn/Al LDHs	Fibroblast 3T3 cells and human normal lung cells (MRC-5)	Saifullah et al ¹²³
8	<i>Para</i> -aminosalicylic acid ZnLH	Fibroblast 3T3 cells and human normal lung cells (MRC-5)	Saifullah et al ¹⁵³
9	Isoniazid–Mg/Al LDHs	Fibroblast 3T3 cells and human normal lung cells (MRC-5)	Saifullah et al ¹⁵⁴
10	Isoniazid–Zn/Al LDHs	Fibroblast 3T3 cells and human normal lung cells (MRC-5)	Saifullah et al ¹⁵⁵
11	Cinnamic acid ZnLH	Fibroblast cells (3T3)	Mohsin et al ⁴²
12	Cinnamic acid into Zn/Al LDHs	HDF cells	Mohsin et al ¹⁰¹
13	Benzophenone-4–Zn/Al LDHs	HDF cells	Mohsin et al ¹⁰¹
	Eusolex® 232–Zn/Al LDHs	HDF cells	Mohsin et al ¹⁰¹
14	Benzophenone-9–Zn/Al LDHs	HDF cells	Mohsin et al ¹⁰¹
15	Ellagic acid ZnLH	Normal human breast epithelial MCF-10A cells and murine fibroblast 3T3 cells	Hussein-Al-Ali et al ¹⁸⁵
16	Levodopa and Zn/Al LDHs	PC 12 cell model	Kura et al ¹⁷⁸

Abbreviations: NP, nanoparticle; ZnLHs, zinc layered hydroxides.

- 1) coprecipitation (direct method)
- 2) ion exchange (indirect method)
- 3) reconstruction (memory-effect method)
- 4) sol–gel synthesis
- 5) preparation of LHS.

Coprecipitation (direct method)

A coprecipitation technique is most commonly applied for direct one-pot synthesis of LDHs with a variety of divalent and trivalent cations and different anions ranging from inorganic anions, such as Cl^- , NO_3^- , and CO_3^{2-} and a variety of organic molecule dyes and even large biomolecules have been intercalated.^{1,2,8,10,11} Moreover, coprecipitation methods can be applied for the large-scale production of the material of interest.²

In coprecipitation, both the divalent cations and trivalent cations along with the anions to be intercalated are mixed in aqueous solution, and pH is increased by adding basic solution.^{2,18} Coprecipitation is often the preferred method of choice over other methods, as most of the anions can easily

be intercalated using this method.² The coprecipitation of cations is ensured by applying a supersaturation condition, which can be easily reached by controlling the pH of the solution.¹⁵ Thermal treatment is often performed after coprecipitation to ensure maximum yield and to improve the crystallinity of the sample. Thermal treatment can be done by aging the solution at 25°C–100°C. Alternatively, it can be performed by hydrothermal methods, wherein the sample is subjected to high pressure ranging from 10 to 150 MPa and high temperature in autoclave bombs with duration ranging from a few hours to a few days.^{2,19,20}

Ion exchange (indirect method)

Ion exchange is also commonly used for LDH synthesis, and has been successfully applied for the intercalation of a number of different types of anions.^{1,2,8,11} It is also known as an indirect method, in which the first LDHs are prepared with host anions, most commonly NO_3^- , CO_3^{2-} , and Cl^- . In the later stages, anions present in the interlayer region are exchanged with the desired anions.^{1,2} The host–guest

Table 3 In vivo biocompatibility of layered double hydroxides (LDHs) using animal models

Sample	Type of LDH	Animal model	Reference
1	Indomethacin–Mg/Al LDHs	Both sexes Swiss mice	Del Arco et al ¹⁸⁷
2	Ketoprofen–Mg/Al LDHs	Mice	Silion et al ¹⁹²
3	Ketoprofen–Zn/Al LDHs	Mice	Silion et al ¹⁹²
4	Acetylsalicylic acid–dextran LDHs	Rabbits	Dong et al ¹⁶⁰
5	LDH nanoparticles	Mice	Yu et al ¹⁹³
6	Mg/Al LDH nanoparticles	Mice	Flesken-Nikitin et al ¹⁹⁴
7	Levodopa–Zn/Al LDHs	Rats	Kura et al ¹⁹⁵

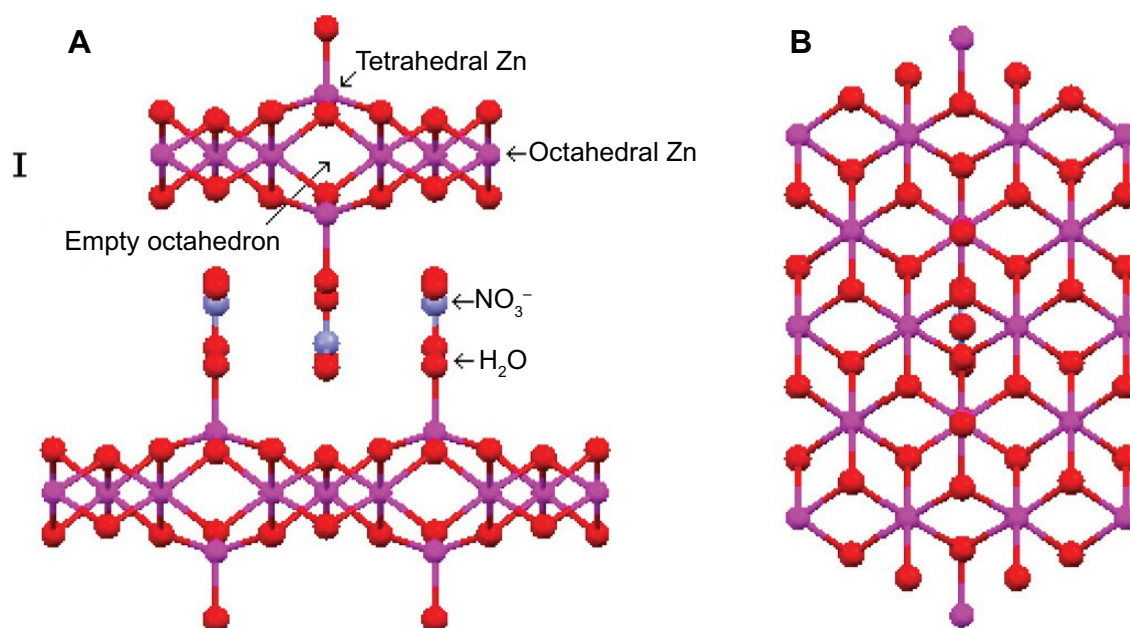
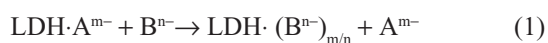


Figure 4 Structures of zinc hydroxide nitrate.

Notes: (A) Side view and (B) top view. Reprinted from Arizaga GG, Satyanarayana KG, Wypych F. Layered hydroxide salts: synthesis, properties and potential applications. *Solid State Ionics*. 2007;178:1143–1162, Copyright 2007, with permission from Elsevier.¹¹

exchange mainly depends on the electrostatic forces between positively charged LDH layers and the exchanging anions, and Figure 5 shows the typical ion-exchange process.²¹

Anions of interest can be intercalated in two possible ways, as described in the equations:



or



In the first example, the anions to be deintercalated are univalent, such as Cl^- , NO_3^- , or ClO_4^- . There exists weak

electrostatic interaction with layers, and hence these anions can be easily replaced by anions with higher electrostatic interaction with layers.

In the second example, there is strong interaction between the positive layers and anions, as they have higher negative charge, such as CO_3^{2-} or carboxylate. But these divalent anions are susceptible to acid attack, and can be easily exchanged with univalent anions, such as (Cl^-), (NO_3^-), or (ClO_4^-).^{1,2} Different anions have been intercalated by ion-exchange methods, such as the inorganic anions Cl^- , NO_3^- , SO_4^{2-} , CrO_4^{2-} , HAsO_4^{2-} , HPO_4^{2-} , and CO_3^{2-} , pharmaceutical agents like *para*-aminosalicylic acid (PAS), and cetirizine.^{1,2,22–25}

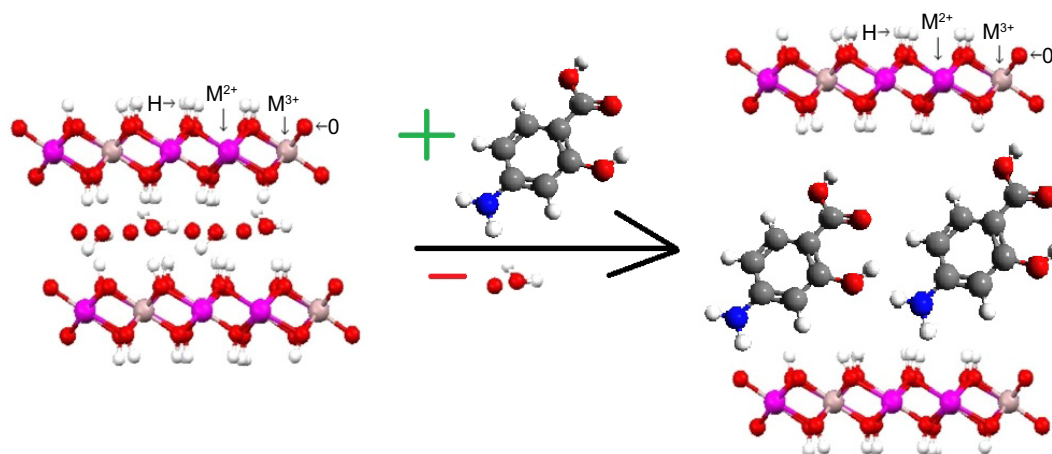


Figure 5 Schematic representation of ion-exchange process.

Regeneration of layered double hydroxides by structural memory effect

LDHs can be completely transferred to the respective metal oxide by heating the LDHs at high temperature, a process known as calcination. In this method, LDHs are heated at between 500°C and 600°C. As a consequence, interlayer water molecules, intercalated anions, and hydroxyl groups of LDHs are completely removed, leaving behind mixed metal oxides.^{23,26–28} It should be noted that preferably LDHs should be heated at about 400°C–550°C; heating at higher temperature will affect the regeneration of LDHs.^{2,18} When these mixed metal oxides are exposed to water and anions of interest, LDHs are reformed. However, due to the structural memory effect, if only the metal oxides that are not formed from LDHs are used, they will not generate LDHs.^{2,18,26–28} Calcination and anions of interest can be introduced after calcination and rehydration process.^{2,18} This is a very useful method, especially for the intercalation of organic molecules, and many different anions have been successfully incorporated in LDHs, such as caprolactam, organic chromospheres, dyes, surfactants, and metal complex anions, and this is even a very useful method for the intercalation of nonionized species, eg, sugar molecules like pentoses.^{29–33} The whole process of rehydration should be carried out under an inert nitrogen atmosphere.^{2,18}

Hydrothermal method

In the hydrothermal method, metals either in hydroxide forms or in the form of salts or anions of choice are mixed, and the pH of the solution is raised to the desired level. For Mg/Al LDHs, for example, the pH is usually set at 9–10 and for Zn/Al LDHs, the pH is set at 7–7.5.^{20,34,35} After rising the pH, the sample solution is enclosed in stainless steel autoclave hydrothermal bombs, then the sample is either heated to a certain temperature ranging from 50°C to 180°C or it is subjected to simultaneous rotations.^{34–36} This method is particularly useful for the intercalation of anions with low affinity for LDHs compared to the competing anions of starting salts, such as Cl^- and NO_3^- .² Intercalation of low-affinity anions into LDHs using the hydrothermal procedure can be more effective by the application of insoluble hydroxides, such as magnesium hydroxides and aluminum hydroxides, instead of their salts, which contain counteranions like Cl^- and NO_3^- .³⁶ By this method, very fine LDHs are formed. Zhao et al synthesized very fine nickel/aluminum LDH nanorods with a size range of 15–40 nm.³⁴ By this method, properties of LDHs can easily be tuned by controlling the temperature, pressure, and contact time.³⁵

Preparation of metal layered hydroxides

Metal layered hydroxides (layered hydroxyl salts [LHS]) can be prepared in two ways: by using salt of the chosen metal and by using metal oxide.^{37–39} In the first method, salt is used as the starting material, which is dissolved in aqueous solution and its pH is increased by the slow addition of basic solutions, such as sodium hydroxides, aqueous solution of ammonia, or aqueous carbonate, until precipitation occurs.⁴⁰ This method is very much similar to the coprecipitation method used in LDH preparation, where aqueous salt solutions are coprecipitated with counteranions of interest.^{23,24}

Metal layered hydroxides can also be prepared using metal oxide as the starting material. In this method, metal oxide is dissolved in the solution of the desired anions, and the pH is raised until precipitation occurs.^{37,39} The results are more fruitful if the desired anion solution is acidic in nature.^{37,39} Varieties of organic and inorganic anions have been intercalated using these two methods.^{39–42}

Application of layered double hydroxides

Catalytic

LDHs have been used as catalysts as well as catalyst support in a number of reactions.⁴³ They have been applied in the oxidation of ethanol, methanol, selective oxidation of glycerol, selective oxidative halogenations, and oxidation of water for oxygen production and several other applications.^{43–49}

Environmental remediation

LDHs are considered to be an ideal material for the removal of toxic material from water and also from the atmosphere.^{50,51} Motor vehicles, such as cars and motorcycles, are now integral to modern requirements. However, their increased use has also deteriorated our environment, due to the toxic gases being released as a by-product of combustion. Nitrogen oxides and soot exhaust from our vehicles are the most common causes of environmental and health problems.⁵⁰ LDHs with different metal compositions have been applied in the adsorption of nitrogen oxides and soot.^{52–57} Removal of toxic material, both organic and inorganic, is very difficult task, which LDHs have successfully accomplished.⁵⁸

Moreover, LDHs have been successfully applied in the removal of toxic metals in the form of their oxides, such as selenium, arsenic, chromium in wastewater, and radioactive uranium (VI), and have also been utilized for the removal of other heavy metals.^{58–61} Also, halogen anions and perchlorate anions have been removed by LDH application.^{62–65} Organic

toxins are very difficult to remove from water, but this has been rendered possible by LDH application. Another study on the removal of guar gum and humic acid demonstrated very positive removal from water with the application of LDHs.⁶⁶ LDHs have also been successfully utilized in the removal of many organic compounds from water, such as *N,N*-dimethyl aniline, 2-chlorophenol, phenolic compounds, dodecylbenzenesulfonate, and Remazol Blue 19. Even bacteria and viruses have been removed from water by the application of LDHs.^{32,67–70}

In water-treatment plants, disinfection is an important procedure, and currently halogens and ozone are employed as disinfectants. However, there are certain issues with respect to these disinfectants, such as the fact that they can form genotoxic and carcinogenic by-products in the presence of organic compounds.⁷¹ Nowadays, scientists are looking for alternative disinfectants. One of the important disinfectants is lysozyme (LYZ), also called muramidase, which can break down the bacterial cell wall, resulting in bacterial eradication.⁷² Yang et al developed a novel disinfectant by the intercalation of LYZ into LDHs (LYZ LDHs), and its antibacterial capability was evaluated against *Staphylococcus aureus*. The antibacterial activity of LYZ LDHs was found to be consistently above 94% in the pH range of 3–9, which was much higher compared to free LYZ. This was due to the double action of LYZ LDHs, ie, adsorption of LDHs and antibacterial action of LYZ.⁷³ The LYZ LDH formulation is considered to be a green antibacterial agent, as it does not produce any by-product.

Flame retardancy

One of the important characteristics of LDHs is their high thermal stability, which makes them an ideal halogen-free retardant material.⁷⁴ They have been applied alone and in combination with polymers for fire control.^{74,75} Gao et al developed flame-retardant nanocomposite-based LDHs with different anions like nitrate, carbonate, chloride, and sulfonate, and combined them with a high-density polyethylene polymer.⁷⁵ There are many other LDHs and polymer composites that have been developed and applied as flame retardants.^{74,76–79}

Layered double hydroxides in biosensing

Biosensing is an emerging technology that has a number of applications in biomedical diagnosis, food sciences, and environmental sciences. The sensors are small devices that can detect chemical or biochemical changes in the medium around them and can convert them into the analytically

useful signal. The sensor contains two basic components: 1) a receptor that can recognize the occurrences of events in the analytes of interest and 2) a transducer that converts the events into measurable signals.⁸⁰ In biosensors, transducers are attached with biological materials (enzymes, affinity ligands, antibody, oligonucleotides, receptors, peptides, etc), which can serve as a biological recognizer.⁸¹

The high thermal stability and tendency of inorganic nanolayers to protect immobilized bioactive molecules and their biocompatibility make them ideal material to be applied in biosensing technology. In this section, the application of LDHs in biosensing technology is highlighted.

Layered double hydroxide-based chemiluminescence flow cell sensor

Zhang et al designed a chemiluminescence (CL) flow cell sensor by immobilizing Co(II)-ethylenediaminetetraacetic acid (EDTA)-intercalated Mg/Al LDHs on a clear quartz tube. The developed microcell enhanced the CL signal from the luminol–H₂O₂ reaction, and has the strong potential to be applied in chemical/biological sensing for H₂O₂, as well as for other oxidase-based reactions producing H₂O₂. The limit of detection for H₂O₂ was found to be 0.14 μM with a signal-to-noise ratio of 3 (S/N = 3).⁸²

The likely mechanism of CL in the enhanced luminol–H₂O₂ system-based Co(II)-EDTA complex-intercalated LDHs is shown in Figure 6. There is an equilibrium between the Co(II) and EDTA, accompanied by a constant source of Co(II) ions to the luminol–H₂O₂ system. The Co(II) ions released then react with H₂O₂, producing lots of OH[–] and O^{2–} ions, which can further react with luminol to emit light.^{82–84}

Hemoglobin and DNA-fabricated layered double hydroxide-based biosensor

Liu et al designed a biosensor by fabricating hemoglobin, deoxyribonucleic acid (DNA), and Ni/Al LDHs. The hemoglobin-DNA LDH-based biosensor showed sensitivity toward H₂O₂ and NO₂ in the linear range of 4.85–10^{–7} to 1.94–10^{–4} M and 2.5–10^{–7} to 3.0–10^{–5} M, respectively.⁸⁵

Acetylcholinesterase: layered double hydroxide-based biosensor

Gong et al designed highly sensitive amperometric biosensors for the biosensing of organophosphate pesticides by the fabrication of LDHs with acetylcholinesterase (AChE). They found that LDHs facilitate a biocompatible microenvironment that assists in maintaining the bioactivity of AChE because of the intrinsic properties of LDHs, specifically regular structure, good chemical, mechanical, and thermal

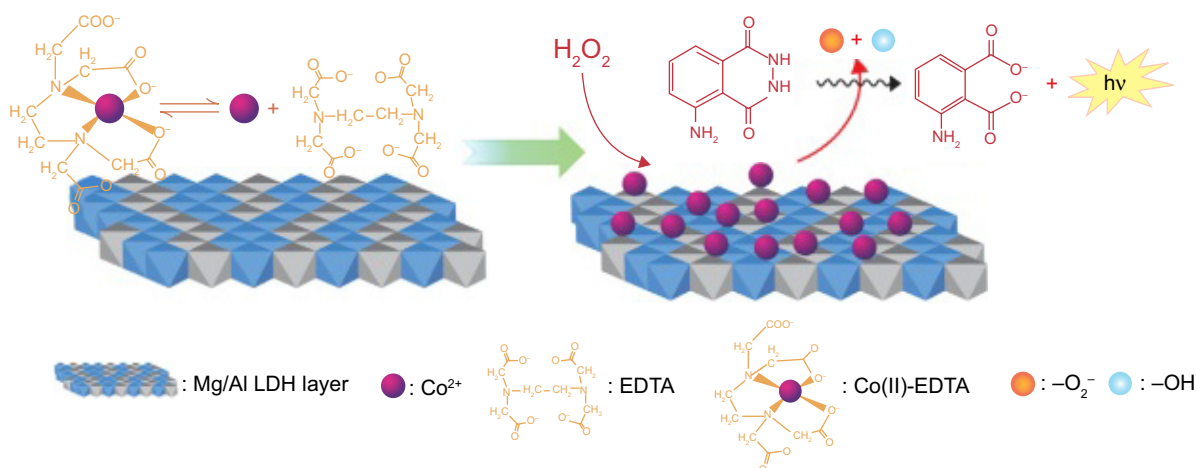


Figure 6 Possible chemiluminescence mechanism for the Co(II)-ethylenediaminetetraacetic acid (EDTA)-intercalated Mg/Al layered double hydroxide (LDH)-enhanced luminol-H₂O₂ system.

Note: Reprinted from Zhang LJ, Chen YC, Zhang AM, Lu C. Highly selective sensing of hydrogen peroxide based on cobalt-ethylenediaminetetraacetate complex intercalated layered double hydroxide-enhanced luminol chemiluminescence. *Sens Actuators B Chem.* 2014;193:752–758, Copyright 2014, with permission from Elsevier.⁸²

stability, and swelling properties. They found that AChE LDH-based electrodes greatly enhanced catalysis, oxidation producing thiocholine, and facilitated improved sensitivity with a detection limit of 0.6 ng·mL⁻¹ (S/N=3).⁸⁶

Luminol-layered double hydroxide-based biosensor for glucose detection

Wang et al developed a biosensor by the fabrication of Mg/Al LDHs with luminol for glucose detection in human serum. They combined the Mg/Al LDH–luminol with silica gel-fabricated glucose oxidase. The concentration was determined by CL, which can detect glucose in a linear range of 0.005–1.0 mM. The detection limit for glucose was found to be 0.1 mM, with S/N=3.⁸⁷ Colombari et al designed a biosensor for the amperometric detection of glucose by combining Mg/Al LDHs with glassy electrode carbon.⁸⁸

Ionic liquid: layered double hydroxide-based biosensor for protein detection

Lou et al developed a biosensor based on ionic liquid 1-carboxyl-methyl-3-methylimidazolium tetrafluoroborate (CMMIMBF₄) and LDHs for redox protein detection. The designed biosensor facilitated faster electron transfer in the redox process and was highly suitable for the detection of redox proteins.⁸⁹

Gold-layered double hydroxide-based biosensor for toxic nitrite compound

Nitrite is the most commonly found compound in foods and the environment. It has been reported to be toxic and carcinogenic, and can even block oxygen supply via the blood

by irreversible oxidation of hemoglobin to methemoglobin.^{90,91} Yin et al developed a biosensor by the fabrication of the gold electrode with Cu-Mg-Al LDHs, which were able to detect the nitrite amperometrically. The designed LDH-based biosensor showed excellent bioelectrocatalytic activity for nitrite oxidation in a linear range of 0.75–123 μM, with a detection limit of 2×10⁻⁷ M and S/N=3. The biosensor successfully determined nitrite content in food samples.⁸¹

Layered double hydroxide-based biosensor for cysteine sulfoxide detection in food samples

Anifantaki et al designed a biosensor by fabricating alliinase with LDHs for cysteine sulfoxide detection. The developed biosensor has the strong potential to be applied in food science.⁹² Mansouri et al designed a conductometric biosensor for polypeptide biosensing by the intercalation of trypsin into Zn/Al LDHs and Mg/Al LDHs.⁹³

Layered double hydroxides in bioimaging

Bioimaging is one of the latest scientific techniques applied for disease diagnosis and examination of disease, and is also being applied in medical science to study normal physiology and anatomy. In bioimaging techniques, different processes are used to develop human body image, tissues, and anatomical area down to the molecular level.

LDHs can be ideal material to be applied in bioimaging science, due to their proven high biocompatibility (both in vitro and in vivo studies), positively charged surface, and ease of interaction with negatively charged cell membranes, which

improve the efficiency of transfer.⁹⁴ LDHs are most recently explored in the bioimaging science and only few studies are carried out using LDHs in bioimaging science.

Layered double hydroxide-based upconversion fluorescence for bioimaging and drug delivery in cancer therapy

Chen et al designed a multifunctional nanodelivery system for drug delivery to and bioimaging of tumors by the fabrication of $Y_2O_3:Er^{3+}, Yb^{3+}$ nanoparticles (NPs; near-infrared fluorescent nanophosphors) and fluorouracil (5-FU; anticancer drug) intercalated into Mg/Al LDHs, collectively abbreviated as $(Y_2O_3:Er^{3+}, Yb^{3+}@SiO_2@LDH-5FU)$.⁹⁵ The developed nanodelivery systems showed powerful red upconversion fluorescence upon excitation with a 980 nm laser, which permitted the easy tracking of the nanodelivery system after internalization in cancer cells. The resultant nanodelivery system showed better anticancer efficacy and stronger red fluorescence, as shown in Figure 7. The better therapeutic outcome with the nanodelivery system compared to the free 5-FU can be attributed to the better internalization of positively charged NPs (due to LDHs' positive surface) in the cell membrane.⁹⁵

Layered double hydroxide-based multifunctional core/shell nanospheres for bioimaging and targeted delivery in cancer

Li et al designed novel biodegradable multifunctional core/shell nanospheres with targeted, controlled release and

fluorescent properties for cancer therapy. First, they prepared a core based on iron magnetic supraparticles (MSPs), which were then coated with a shell of Ni/Al LDH. Next, the doxorubicin (anticancer drug)-carboxyl group, modified (DOX-COOH), was loaded on the shell of MSP LDH nanospheres. In addition, they immobilized the iminodiacetic acid-modified folate over MSP LDH-(DOX-COOH) in order to target cancer cells. Fluorescence microscopy found that the designed nanospheres targeted the cancerous cells and were highly cytotoxic to the cancerous cells compared to normal HEK 293T cells.⁹⁶

Layered double hydroxide-based luminescent systems for bioimaging

Yan et al designed a highly luminescent nanocomposite by the fabrication of sodium fluorescein dye with Mg/Al LDHs. The nanocomposite was found to exhibit excellent fluorescence intensity, maintain fluorescence even in the form of dry powder, and was able to form transparent free-standing film (fluorescence active in ultraviolet [UV] light).

Tanaka et al intercalated a fluorescent compound fluorescein (Fluo) into Mg/Al LDHs and studied their internalization and intracellular behavior in mammalian cells. They found that the Fluo LDHs displayed high green fluorescence in whole cells, including the nucleus. Furthermore, the study revealed that anion fluorescein was released from Fluo LDHs and endosomes by the proton-assisted dissolution of Fluo LDHs.⁹⁷

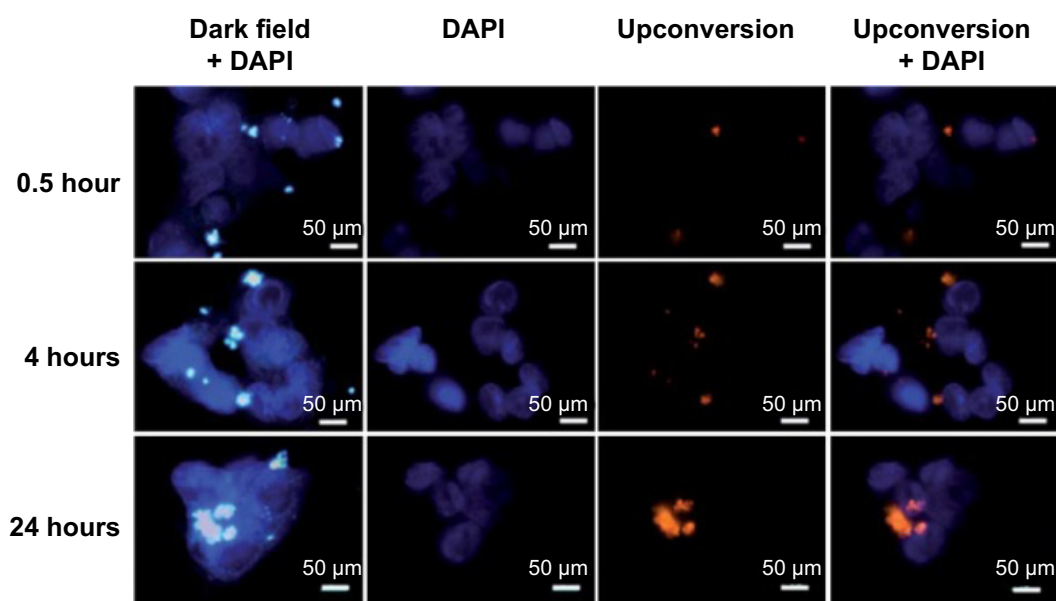


Figure 7 CytoViva microscopy images of MCF-7 cells incubated with $Y_2O_3:Er^{3+}, Yb^{3+}@SiO_2@LDH-5-FU$ at $100 \text{ mg} \cdot \text{mL}^{-1}$ for different time durations of 0.5 hour, 4 hours, and 24 hours.

Note: Reproduced from Chen C, Yee LK, Gong H, Zhang Y, Xu R. A facile synthesis of strong near infrared fluorescent layered double hydroxide nanovehicles with an anticancer drug for tumor optical imaging and therapy. *Nanoscale*. 2013;5:4314–4320, with permission of The Royal Society of Chemistry.⁹⁵

Abbreviations: DAPI, 4,6-diamidino-2-phenylindole; LDH, layered double hydroxide; 5-FU, fluorouracil.

Musumeci et al fabricated LDHs with different organic dyes for understanding the biological interactions and their biocompatibility with LDHs. They intercalated LDHs with organic dyes, namely fluorescein isothiocyanate (FITC), fluorescein, 8-aminopyrene-1,3,6-trisulfonic acid, and 8-aminonaphthalene-1,3,6-trisulfonic acid. Confocal imaging after incubation of LDH-dye nanocomposites with cells revealed that for biological tracking and labeling purposes, organic dyes were suitable in the following order: 1) fluorescein, 2) sulfonic acid-derived pyrene, and 3) naphthalene-based dyes.⁹⁸

Posati et al doped Zn/Al LDHs with europium (III) ions via microemulsion methods, and luminescence measurements revealed that the resulting material, europium (III)-Zn/Al LDHs, was found to be very luminescent.⁹⁹

Layered double hydroxides in UV protection and sunscreen formulations

Most skin cancers are caused by solar UV radiation, and skin cancer is responsible for human deaths. According to the World Health Organization, about 60,000 people died from cancer in the year 2000.¹⁰⁰ We are exposed to sunrays every day; visible light is beneficial for us, but at the same time we are also exposed to UV (carcinogenic) radiation. Sunscreen formulation is the most commonly applied defense against UV radiation, but these sunscreen formulations contain organic UV absorbers that have certain limitations, eg, they cannot cover the whole UV region (200–400 nm). These organic UV absorbers undergo photodegradation under UV radiation, and the degradation products are toxic.⁴² Therefore, there is a need to design new sunscreen protection formulations free from the aforementioned demerits. The photostability of these organic UV absorbers can be achieved by intercalating them with inorganic nanolayers. Inorganic nanolayers are highly biocompatible, and most importantly they also absorb in the UV region, resulting in a hybrid material to cover the larger UV region (200–400 nm).

Mohsin et al designed an efficient sunscreen formulation by the intercalation of an organic UV absorber – cinnamate anion – into zinc layered hydroxides (ZnLHs). The developed formulation covered the whole UV region, and was found to be biocompatible with HDF cells. In addition, cinnamate anion was thermally stabilized in the interlayers of ZnLHs.⁴²

Mohsin et al also recently designed three UV-absorbing nanocomposites by separate intercalation of three organic UV absorbers, namely cinnamic acid (CA), benzophenone-4, and Eusolex® 232, into Zn/Al LDHs. The study revealed that the UV-absorption range of all of the nanocomposites was longer

compared to the free individual organic absorbers. Figure 8 shows the UV-absorption spectrum of CA, Zn/Al-CA, and Zn/Al LDHs. It can be easily seen that Zn/Al-CA covered a larger UV region compared to the CA alone. Likewise, the UV absorption of the two other nanocomposites showed similar trends. Most importantly, a cytotoxic study revealed that all three nanocomposites were found to be biocompatible with HDF cells.¹⁰¹

Mohsin et al have recently also developed a UV-absorbing nanohybrid (sunscreen formulation) by intercalating the strong UV absorber benzophenone-9 into Zn/Al LDHs. The sunscreen formulation was found to be stable in deionized water, artificial seawater, and in skin pH conditions. The UV-absorption spectrum was found to be broader compared to the free benzophenone-9, and the nanocomposite was also determined to be biocompatible with HDF cells.¹⁰²

Wang et al designed novel UV-absorbing thin film by doping Fe³⁺ with Mg/Al LDHs, and the resulting material covered the whole UV region (200–400 nm). Furthermore, the thin film was easily fabricated with highly transparent polymer polysiloxanes and UV-blocking composite material. The designed transparent UV-blocking composite can potentially be applied in protective coatings for glass curtain walls, automotive glass, outdoor wood objectives, priceless scrolls of calligraphy, cultural relic paintings, and some UV-sensitive materials.¹⁰⁰

Li et al designed a UV-absorber organic–inorganic nanocomposite by the intercalation of 2,4-dihydroxybenzophenone into dodecylbenzenesulfonate-fabricated LDHs. The UV-absorption capability of the designed nanocomposite

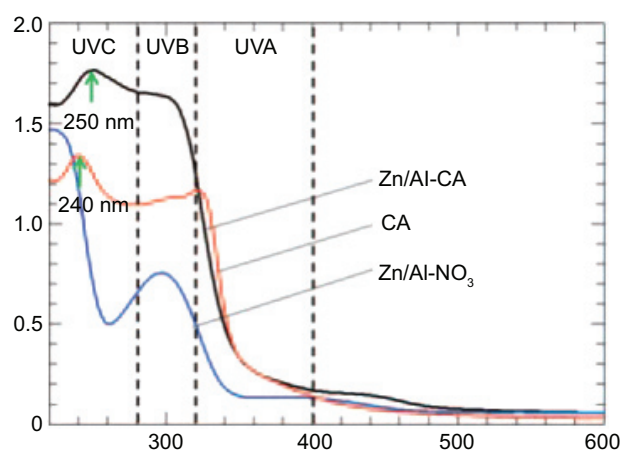


Figure 8 Solid-state absorbance spectra of Zn/Al-NO₃, organic sunscreen guest cinnamic acid (CA), and the corresponding nanocomposite.

Note: Reprinted with permission from Mohsin SM, Hussein MZ, Sarijo SH, Fakurazi S, Arulselvan P, Taufiq-Yap YH. Characterisation and cytotoxicity assessment of UV absorbers-intercalated zinc/aluminium-layered double hydroxides on dermal fibroblast cells. *Sci Adv Mater*. 2014;6:648–658. Copyright © American Scientific Publishers.¹⁰¹

Abbreviation: UV, ultraviolet.

was extended over the entire UV region, which suggests it can be potentially applied as a UV absorber.¹⁰³

Drug-delivery applications of layered double hydroxides

LDHs have been the focus of several researchers, due to their potential applications in the biomedical field, and the research trends have proved that LDHs are the ideal material for this field. In this section, recent advances in drug-delivery application for the cure of various diseases are discussed.

Also in this section, the latest developments in the drug-delivery applications of inorganic nanolayers are discussed. Many nonsteroidal anti-inflammatory drugs have been intercalated into inorganic nanolayers; Rives et al comprehensively reviewed the application of LDHs to the delivery of nonsteroidal anti-inflammatory drugs.⁸ Many other drugs, such as antidiabetes, cardiovascular, antibiotics, antioxidants, antiosteoporosis, vitamins, amino acids, and peptides, have been intercalated into inorganic nanolayers, which were comprehensively reviewed by Rives et al recently.⁷

Layered double hydroxides in anticancer formulations

Cancer has been threatening humans for centuries, and despite technological advancements, cancer claims millions of human lives every year, with about 8.2 million deaths in 2012.^{104,105} The statistics of increasing cancer cases suggest that by the year 2025, cancer-related deaths will increase by up to 80% in less developed countries.¹⁰⁴ Cancer is a set of diseases caused by the uncontrolled growth of abnormal cells and spread of these abnormal cells. It has been reported that if the spread of these abnormal cells is not controlled, they ultimately lead to death.¹¹⁰ Of drugs, such as decrease in bloodstream cell count, hair loss, cardiac dysfunction and heart failure, hyperglycemia, bone marrow depression, vomiting, nausea and diarrhea, tiredness, mouth soreness, constipation or diarrhea, loss of appetite, skin changes or reactions, pain or nerve changes, and changes in fertility and sexuality.^{106–109} Resistance of cancer cells toward effective therapeutic drugs is another major issue in cancer therapy.^{110,111}

Drug-delivery systems could be very handy in reducing the adverse side effects of anticancer drugs, and could target cancer cells and release the drugs in a sustained manner. All of these factors would enhance therapeutic efficacy.

Methotrexate layered double hydroxides

Methotrexate (MTX) is an anticancer drug that can deactivate the metabolism of diseased cells effectively via programmed cell death (apoptosis), and is being used against different human cancers, eg, osteosarcoma (bone cancer) and leukemia.¹¹² Jae-Min et al evaluated the anticancer efficacy of free MTX and MTX LDHs against bone cancer cell lines (Saos-2 and MG-63), and found that MTX LDHs were more effective in suppressing the cancer cells compared to free MTX. Oh et al revealed that LDHs were highly cytocompatible with human fibroblast cells even up to 500 $\mu\text{g}\cdot\text{mL}^{-1}$.¹¹³

Zhang et al recently chose MTX LDHs as a model nanohybrid to study the effect of different hydrothermal conditions on particle size and the effect of particle size on control release and suppression of cancer cells.¹¹⁴ They found a direct relationship between the diameter of LDHs and prolonged hydrothermal treatment at higher temperatures. In addition, hydrothermal treatment had no effect on percentage drug loading.¹¹⁴ Furthermore, they studied the effect of particle size on in vitro drug release, and found a direct relation between the size of LDHs and sustained release: the bigger the size, the longer the release. They considered the shelf life of MTX LDHs, and found that release was excellent even after 18 months, which makes LDHs suitable for drug delivery.¹¹⁴

Ifosfamide layered double hydroxides

The intercalation of neutral or nonionic molecules into LDHs is very difficult and challenging, because the positive layers of LDHs have very low affinity for neutral molecules. Alternative methods for neutral species intercalation are either the memory-effect method or the direct assembly of neutral molecules with negatively charged surfactants like sodium dodecyl sulfate, sodium dodecylbenzenesulfonate, cholate, and deoxycholate.

Ifosfamide (IFO) is an anticancer agent commonly applied for the treatment of many pediatric and adult tumors, including soft-tissue sarcomas and lymphomas.^{115,116} IFO is a nonionic drug, and nonionic drugs are difficult to intercalate into LDHs. Nie and Hou intercalated IFO into Mg/Al LDHs by the surfactant-assisted method, in which they applied sodium dodecyl sulfate and sodium dodecylbenzenesulfonate.¹¹⁷ They intercalated IFO into surfactant-modified Mg/Al LDHs separately. They conducted in vitro release in phosphate-buffered saline (PBS, pH 7.5), and found that IFO release was much more sustained from the LDH-surfactant-IFO nanohybrid compared to the physical

mixture of free IFO and LDHs. The kinetic study of IFO release from LDHs was revealed to follow a pseudo-second order process.¹¹⁷

Camptothecin-layered double hydroxides

Camptothecin (CPT) is highly potent against a variety of cancers, such as lung cancer, ovarian cancer, pancreatic cancer, and stomach cancer.^{118,119} However, the chemical instability of CPT and its poor water solubility limit its clinical applications.^{118,119}

Recently, Wu et al successfully intercalated the neutral anticancer drug CPT into Mg/Al LDHs by coassembling it with sodium cholate, and then intercalated the CPT-sodium cholate by the delaminating method.¹²⁰ The loading of CPT was found to be 13%, and PBS solution of pH 7.4 was found to be much more sustained compared to the physical mixture of CPT and sodium cholate LDHs.¹²⁰ In addition, they also determined release kinetic models, which satisfactorily adopted the parabolic kinetic model, which suggests that release possibly follows a diffusion mechanism. However, ironically, they did not study the anticancer effect, which is crucially important for drug-delivery applications.

Protocatechuic acid layered double hydroxides

Protocatechuic acid (PA; 3,4-dihydroxybenzoic acid) is an anticancer agent widely used for the treatment of different types of cancers, namely cervix, breast, human leukemia (pa-2000-leukemia), liver, lung, and prostate cancers.¹²¹ Barahuie et al intercalated PA into Mg/Al LDHs (PA-Mg/Al LDHs) by coprecipitation and ion-exchange methods, and studied various drug-delivery aspects.¹²² Anticancer assay revealed that PA-Mg/AL LDHs had higher anticancer activity compared to the free PA against the HeLa cervical cancer cell line and MCF-7 breast cancer cells. In addition, nanocomposites did not show any cytotoxicity to normal 3T3 fibroblast cells. The in vitro release study revealed that PA release from PA-Mg/Al LDHs was much more sustained compared to their physical mixture.¹²² The in vitro release of PA from Mg/Al LDHs was found to follow a second-order kinetic model more satisfactorily.¹²²

Etoposide layered double hydroxides

Etoposide (VP16) is a derivative of podophyllotoxin, and has been reported to have significant anticancer activities against various cancer cells, such as small-cell lung carcinoma, gastric cancer cells, hematologic malignancies, childhood malignancies, and germ cell tumors.^{123–127}

Qin et al prepared a nanohybrid formulation of VP16 by intercalating it into Mg/Al LDHs (VP16-Mg/Al LDHs).¹²⁸ They found that the nanohybrid (VP16-Mg/Al LDHs) possessed better antitumor activity against MKN45 and SGC-7901 cells. The release of VP16 from LDHs was found to be sustained and followed a diffusion mechanism, as it followed the parabolic kinetic model.²⁸

Ciprofloxacin zinc layered hydroxides

Latip et al designed a anticancer nanodelivery formulation by intercalating ciprofloxacin into ZnLHs. The release of ciprofloxacin was found to sustain in human body-simulated PBS of pH 7.4. The ciprofloxacin ZnLH nanocomposite showed stronger anticancer effect against A549 cancer cells compared to free ciprofloxacin.¹²⁹ The enhanced anticancer effect can be attributed to the nanoscale size of the nanocomposite and sustained release of the ciprofloxacin. Many other anticancer drugs have been intercalated into LDHs, such as ferulic acid,¹³⁰ cetirizine,¹³¹ ellagic acid (EA),⁴¹ 5-FU-cyclodextrin complex,¹³² and doxifluridine.¹³³

Layered double hydroxides in antimicrobial formulations

Inorganic nanolayers have been applied in designing antimicrobial formulations by intercalating a variety of antimicrobials into them. Here, we discuss some of the latest developments made in the application of inorganic nanolayers in antimicrobial formulations.

Ciprofloxacin layered double hydroxides

Hesse et al intercalated the antimicrobial drug ciprofloxacin into Mg/Al LDHs and evaluated its in vivo antimicrobial effect in rabbit ears against the bacterium *Pseudomonas aeruginosa*, and the material was found to show excellent antimicrobial effect even after 1 week.¹³⁴

Hippuric acid zinc layered hydroxides

Hippuric acid has been reported to have antimicrobial and anti-tumor properties.^{39,135} Hussein Al Ali et al developed nanocomposite formulations by the intercalation of hippuric acid into ZnLHs.¹³⁶ The antimicrobial study revealed that hippuric acid ZnLHs showed strong activity against *P. aeruginosa*, and most importantly hippuric acid ZnLHs showed better antimicrobial properties against drug-resistant bacteria, namely methicillin-resistant *S. aureus*, compared to free hippuric acid.¹³⁶

Benzylpenicillin layered double hydroxides

Wang et al designed an antimicrobial film by intercalating benzylpenicillin (antimicrobial agent) into Mg/Al LDHs, and

then fabricated that nanohybrid with graphene oxide.¹³⁷ They found that sustained release of benzylpenicillin from the film was directly related to the thickness of the film, and the film showed strong antimicrobial activity against *Micrococcus lysodeikticus* and sulfate-reducing bacteria.¹³⁷

Amino acid layered double hydroxides

Wang et al designed antimicrobial formulations by intercalating different complex amino acids based on the Schiff base ligands salicylidene with alanine, phenylalanine, glycine, tyrosine, aspartic acid, and glutamic acid for complexes and gallium ion (G^{3+}).¹³⁸ The designed nanocomposites retained antimicrobial activity against *P. aeruginosa*, with a very good minimum inhibitory concentration ($65\text{--}137\ \mu\text{mol}\cdot\text{L}^{-1}$).¹³⁸ Many different antimicrobial agents, eg, amoxicillin¹³⁹ and cefazolin,¹⁴⁰ have been successfully intercalated into LDHs.

Layered double hydroxides as antimicrobial biomaterial

The development of biomaterial with antimicrobial properties is another fascinating area of research, as many diseases are caused by microbes. Biomaterial with antimicrobial properties finds a variety of applications, such as in medical implants, medical devices, food packaging, and household products.¹⁴¹ Inorganic nanolayer composition based on metals is renowned for its antimicrobial effect. Some compounds, such as silver, zinc, and copper, can be very useful for the production of different LDH-based ceramic materials in floor tiles, kitchens, and even bathrooms.¹⁴² In this section, we discuss applications of LDH-based biomaterial as antimicrobial agents.

Silver-based layered double hydroxides with antimicrobial properties

Mishra et al incorporated silver into Zn/Al LDHs and compared their antibacterial activity with Zn/Al LDHs, silver-based LDHs, and their calcined product. They found that Ag LDHs before and after calcination remained active against the bacterial species *Escherichia coli* and *S. aureus*.¹⁴² The antimicrobial effect of calcined Ag LDHs (spinel form) would be a useful property that can be applied in household items to prevent microbes, which in itself is an important avenue of research in the field of ceramics. In addition, the antimicrobial drugs intercalated into this type of LDH would result in a synergetic antimicrobial effect.

Biogenic silver nanoparticle-fabricated layered double hydroxides

Silver NPs have been reported to have strong antimicrobial properties, but toxicity associated with them limits their

applications.^{143–145} In order to make silver NPs more biocompatible, Marcato et al fabricated biogenic silver NPs (AgNP_{bio}) with Mg/Al LDHs.¹⁴⁶ They evaluated the biocompatibility of free AgNP_{bio} , LDHs, and AgNP_{bio} LDHs against a lung fibroblast cell line (V79), and found that AgNP_{bio} caused 50% cell death at a concentration of $45\ \mu\text{mol}\cdot\text{L}^{-1}$. However, even at a concentration of $45\ \mu\text{mol}\cdot\text{L}^{-1}$, LDHs alone and AgNP_{bio} LDHs did not show any toxicity.¹⁴⁶ In addition, AgNP_{bio} LDH hybrid material was found to retain the antimicrobial effect, as the minimum inhibitory concentration of AgNP_{bio} remained unchanged against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria: $6.6\ \mu\text{g}\cdot\text{mL}^{-1}$ and $12\ \mu\text{g}\cdot\text{mL}^{-1}$, respectively.¹⁴⁶ This fascinating hybrid material is suitable to be applied in biomedical devices, cosmetics, and household items.

Carja et al previously adopted a similar approach, and developed hybrid material by fabricating AgNPs on the surface of LDHs (AgNP LDHs).¹⁴⁷ They evaluated the antimicrobial effect of AgNPs alone and hybrid AgNP LDHs with respect to time, and found that AgNP LDHs retained the inhibition zone against the Gram-positive *S. aureus* (American Type Culture Collection [ATCC] 25923) and the Gram-negative *E. coli* (ATCC 35218). However, the AgNP zone of inhibition was found to almost disappear with time.¹⁴⁷

Titanium-based layered double hydroxides with antimicrobial properties

Zhao et al designed nanoscale material with photocatalytic activity.¹⁴¹ They developed Zn-Ti LDH nanosheets in the size range of 40–80 nm with a band gap of about 2.3 eV.¹⁴¹ They found that photochemical activity and nanosize confer antimicrobial activity under visible light. The material was found to possess a strong antimicrobial effect against *Saccharomyces cerevisiae* (85% inhibition), *S. aureus* (65% inhibition), and *E. coli* (100% inhibition).¹⁴¹

ZnO nanoparticle-fabricated layered double hydroxides with antimicrobial properties

Zhang et al designed antimicrobial nanocomposite by combining Zn/Al LDHs, waterborne polyurethane, and ZnO NPs. The designed nanocomposite showed strong antimicrobial activity against the Gram-negative *E. coli* and the Gram-positive *S. aureus*.¹⁴⁸

Layered double hydroxides in antituberculosis nanodelivery formulations

Tuberculosis (TB) is an airborne infectious disease caused by the bacterium *Mycobacterium tuberculosis* (MTB). It

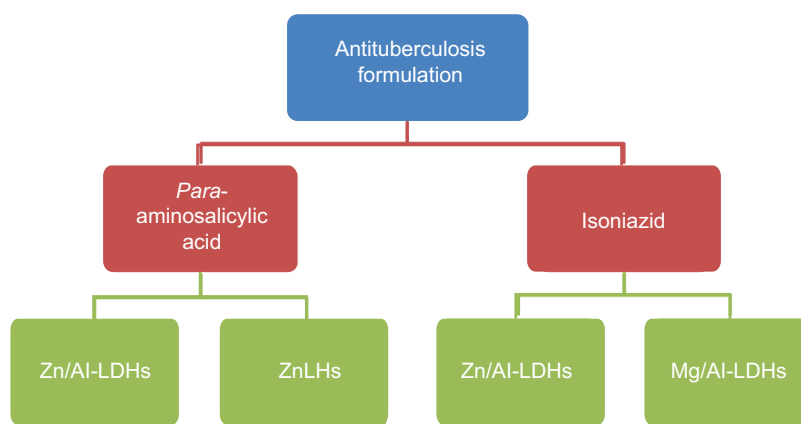


Figure 9 Various antituberculosis nanodelivery formulations based on antituberculosis drugs, para-aminosalicylic acid, and isoniazid with various inorganic nanolayers. **Abbreviations:** LDHs, layered double hydroxides; ZnLHs, zinc layered hydroxides.

can be classified as pulmonary TB when MTB infects the lungs, and the other form is extrapulmonary TB, in which MTB attacks other organs of the human body, namely the liver, kidneys, intestines, bones, tonsils, spleen, and brain.¹⁴⁹ TB has been intimidating humans for centuries, and in the *Global Tuberculosis Report 2013* by the World Health Organization, there were approximately 8.6 million people infected with TB, and about 1.3 million died in the year 2012.¹⁵⁰

There are several issues in TB treatment, such as longer treatment duration (6–24 months), multidrug prescription, frequent dosage of anti-TB drugs, and adverse effects of anti-TB drugs, and all of these factors result in patients' non-compliance with the treatment. Patient noncompliance is the most common reason for the failure of TB chemotherapy.¹⁵¹ There has been no new anti-TB drug that has been introduced in the market, and in a situation like this, biocompatible drug-delivery systems with sustained release seem to be the best option.

Para-aminosalicylic acid layered double hydroxides

Saifullah et al designed an anti-TB nanocomposite formulation with sustained-release properties by intercalation of the anti-TB drug PAS into ZnLHs using zinc oxide as a precursor.³⁷ They have also designed a similar sustained-release PAS ZnLH nanocomposite with higher drug loading by using zinc nitrate as a precursor.¹⁵² Both of these nanocomposite formulations were found to be highly biocompatible with normal human lung fibroblast cells (MRC-5) and 3T3 fibroblast cells. In addition, the nanocomposites showed higher efficacy against bacteria MTB compared to the free drug PAS. The nanocomposites showed antimicrobial activities against *S. aureus*, *P. aeruginosa*, *E. coli*, and *Candida albicans*.^{152,153}

Saifullah et al also reported the development of anti-TB nanocomposite formulations by intercalating PAS into Zn/Al LDHs by coprecipitation and ion-exchange methods.²³ The developed nanodelivery formulation was found to release the PAS in a sustained manner in human body-simulated PBS solutions. They also reported better biocompatibility of the nanocomposites with human normal lung cells (MRC-5) and fibroblast cells (3T3). The nanocomposites were found to be more effective in eradicating MTB compared to free PAS, and were also found to show antimicrobial activities against *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans*.^{23,153} Figure 9 shows the anti-tuberculosis formulations based on inorganic nanolayers and anti-TB drugs.

Isoniazid layered double hydroxides

Saifullah et al recently designed an anti-TB nanodelivery formulation based on the anti-TB drug isoniazid (INH) and Mg/Al LDHs with sustained-release properties. The designed nanodelivery formulation of INH–Mg/Al LDHs was found to be very effective in eradicating the MTB, and most importantly the formulation was found to be more biocompatible when compared to free INH.¹⁵⁴ Saifullah et al also designed another anti-TB sustained-nanodelivery formulation by intercalating INH into Zn/Al LDHs. The designed INH–Zn/Al LDHs showed better therapeutic results against MTB compared to free INH. The as-synthesized INH–Zn/Al LDHs were also found to be more biocompatible with human normal lung cells (MRC-5) and fibroblast cells (3T3).¹⁵⁵ INH-based Zn/Al LDHs and Mg/Al LDHs were also found to show antimicrobial activities against *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans*.^{154,155}

The better therapeutic efficacy against MTB, high biocompatibility with human normal lung cells, and sustained release of the anti-TB drugs will reduce adverse effects as well as dosing frequency, and will shorten the

treatment duration. The sustained-release biocompatible nanodelivery formulation will make TB chemotherapy more patient-friendly.

Layered double hydroxides in gene delivery

The genes are the hereditary units being transferred from parents to children, and are responsible for transporting the characteristics of parents to the children. There are instances where parents with defective genes can pass the disease to their children.¹⁵⁶ Gene therapy is a modern scientific technique that uses genes to prevent and treat disease. It is believed that in the future, this technique will revolutionize medical science in the treatment of all diseases by introducing genes into patient cells instead of drugs and surgery.¹⁵⁷ Scientists are adopting several different approaches in gene therapy, such as the replacement of a defective gene (responsible for disease) with a healthy one, and the introduction of new genes into the body to fight disease.¹⁵⁷ The in vitro and in vivo delivery of bioactive molecules, such as proteins, peptides, and nucleic acids, with the specific focus to transfer these biomolecules into the cytoplasm/nucleus by passing the cell membrane is an important area of biomedical research. There are many issues related to the direct delivery of biomolecules, such as poor solubility and enzymatic degradation. Though there are many hurdles in the development of efficient gene carriers, it is an exciting area of modern biomedical research. Many viral and nonviral carriers have been designed traditionally.¹⁵⁸ If the gene of interest is introduced into the body directly, it

will not work. Therefore, certain carriers are required, which can transfer the gene safely without harming the gene or the human body. Some viruses are currently being tested for the delivery of genes, and these viruses are modified in such a way that they do not harm the body.¹⁵⁷

LDHs offer several advantages over other gene carriers, such as easy preparation, easy intercalation of the biomolecules, targeted delivery, easier removal from the body after delivery without accumulation, high biocompatibility, and the ability to enter the cell via clathrin-mediated mechanism.^{159–161}

Layered double hydroxides in pEGFP-N1-DNA transfection mouse neuron cells

Li et al studied the cellular uptake of LDHs into mouse motor neuron cells (NSC 34) and determined the effect of LDH concentration, size, and incubation time by labeling the LDHs with FITC.¹⁶² The study revealed that cellular uptake was directly proportional to LDH concentration and incubation time. They also found that LDHs of 20 nm size were better at internalizing in the cell cytoplasm and cell nucleus; however, LDHs greater than 20 nm were internalized in the cell cytoplasm.¹⁶² They fabricated LDHs of 20 nm size with plasmid enhanced green fluorescent protein (pEGFP)-N1 DNA, and then successfully transfected them to NSC 34 cells. The found 20 nm LDHs did not cause any cytotoxicity to NSC 34 cells up to 200 mg·mL⁻¹.

Figure 10 shows a confocal image depicting the cytoplasm in green fluorescence after incubation of 6.25 mg·mL⁻¹ of CO₃ LDH-FITC for 2.5 hours. Some of the particles have

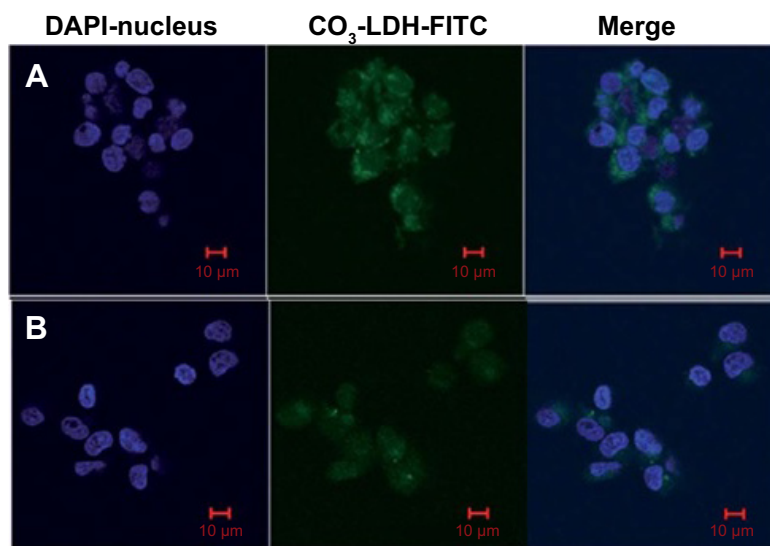


Figure 10 Confocal microscopic images of intracellular localization in NSC 34 cells.

Notes: (A) 6.25 mg·mL⁻¹ CO₃ layered double hydroxide (LDH)-fluorescein isothiocyanate (FITC), incubated for 2.5 hours; (B) free 6.25 mg·mL⁻¹ FITC anions incubated for 4 hours. Reproduced from Li SD, Li JH, Wang CL, et al. Cellular uptake and gene delivery using layered double hydroxide nanoparticles. *J Mater Chem B*. 2013;61–68, with permission of The Royal Society of Chemistry.¹⁶²

also entered the nucleus, as can be seen in green fluorescence in Figure 10A.¹⁶² The free FITC anions showed very weak green fluorescence at a much higher concentration of 6.25 mg·mL⁻¹ compared to CO₃ LDH-FITC. The lower free FITC cytoplasm entrance can be attributed to the mechanism of entrance: free FITC entrance takes place due to diffusion and not by endocytosis.¹⁶²

Layered double hydroxides in plasmid DNA delivery

Hu et al modified the surface of LDHs and studied the effect of surface change on the transfected properties of LDHs. They tailored the LDH surface by fabricating it with 2-(dimethylamino) ethyl methacrylate, and designed a series of surface-modified LDHs for gene delivery.¹⁶³ The surface-modified LDHs showed better tendency to conjugate with plasmid DNA and a higher level of gene delivery in different cell lines, including COS7 and HepG2, compared to free LDHs.¹⁶³

Layered double hydroxides as gene-protective biomaterial

Wu et al investigated the suitability of LDHs as a gene-protective agent in the adverse environment of a Cd²⁺/Pb²⁺ solution.¹⁶⁴ They intercalated DNA into LDHs and subjected both unprotected DNA and LDHs-DNA separately to the Cd²⁺/Pb²⁺ solution. The study revealed that the unprotected DNA structure was damaged, while LDH-protected DNA remained unaffected.¹⁶⁴ Previously, Swadling et al also proved the higher stability of DNA after intercalation by molecular dynamic simulation.¹⁶⁵ Zhang et al studied the structural stability of ribonucleic acid (RNA) before and after intercalation into LDHs by molecular simulation, and their study revealed that RNA had higher stability in LDHs compared to its free form.¹⁶⁶

Codelivery of 5-fluorouracil and siRNAs by intercalation in layered double hydroxides

The emergence of multidrug-resistant cancer is one of the major drawbacks of longer chemotherapy duration for cancer.^{167,168} High-concentration doses are administered to multidrug-resistant cancer patients, causing adverse side effects to healthy tissue.¹⁶⁹ A useful strategy being applied is the coadministration of two different types of anticancer drugs, specifically 5-FU and small interfering RNAs (siRNAs), which have been in use for over 40 years.^{170–172} The sustained and simultaneous codelivery of the 5-FU and siRNAs will improve the therapeutic outcome against cancer.

Li et al designed a novel anticancer formulation by co-intercalation of 5-FU and siRNAs into LDHs for the effective cancer treatment.¹⁷³ The LDH-based sustained codelivery of 5-FU and siRNAs markedly enhanced anticancer activity in comparison to single treatment of either of the two against three cancer cell lines, namely human breast (MCF-7), osteosarcoma (U2OS), and colorectal (HCT-116).¹⁷³ This codelivery formulation has the strong potential to overcome the emergence of drug-resistant cancer.

Transfection of siRNA into cytoplasm by layered double hydroxides

LDHs are suitable vectors to facilitate the uptake and transfection of siRNA, due to their ability to dissolve in an endosomal environment (acidic condition) and buffer the environment.¹⁵⁸ Ladewig et al designed a siRNA-delivery system based on LDHs, and successfully transfected siRNA into the cytoplasm, because of their tremendous ability of escape endosomal environment.¹⁶¹ The cellular uptake study revealed that siRNA LDHs successfully entered HEK293T cells; however, free siRNA failed to enter HEK293T cells. The developed formulation was found to be highly biocompatible with human embryonic kidney cells (HEK 293T) at very high concentrations (up to 0.200 mg·mL⁻¹).¹⁶¹

Many other studies have been conducted on LDHs for their application in gene therapy, such as Ladewig et al (transfected plasmid DNA LDHs into different cells, namely HEK 293T, NIH 3T3, COS-7, and CHO-K1),¹⁶¹ Xu et al (supercoiled pEF-eGFP plasmid LDHs transfected to HEK 293T cells),¹⁷⁴ Hu et al (intercalated mononucleotides and DNA). Table 1 summarizes the biomedical applications of inorganic nanolayers.¹⁷⁵

Sustained release from inorganic nanolayers

The key parameter in designing drug-delivery systems is their tendency to release the active agents (drugs/genes/biomolecules, etc) in a sustained manner at the target site. The tendency of inorganic nanolayers (LDHs/LHS) to release the drug in a desired manner makes them superior to other drug-delivery systems. The drug release from inorganic nanolayers (LDHs/LHS) can be tuned for the desired applications, such as for delivery of the drug in a two-phase manner, initially fast followed by a slower release.^{37,176}

The ability of inorganic nanolayers to protect, release in the desired manner, and release at the target site would avoid the physicochemical degradation of the drug, with a reduction in adverse effects and dosing concentration and frequency,

and in general would improve the bioavailability of the drug. All of these characteristics would improve patients' compliance with treatment, particularly in the management of cancer, TB, and Parkinson's disease, whose drugs have a lot of side effects.^{130,177–179} Here, we discuss the effect of different parameters, such as pH, types of anions present in the release media, and mechanisms involved in drug release from LDHs.

Release in phosphate-buffered saline of pH 7.4 and pH 4.8

In vitro release studies are conducted in various solutions simulating different routes of the delivery. In some cases, the release is carried out in PBS of pH 7.4 to check the suitability of the drug-delivery system for intravenous application, as PBS 7.4 simulates blood.²³ The condition of PBS 7.4 is most commonly applied to evaluate the release behavior of any newly designed drug-delivery system, and the release of different drugs from LDHs/LHS have been conducted in PBS 7.4. The drug release in PBS 7.4 from LDHs/LHS has been found to be highly sustained compared to acidic pH conditions.^{22,24,37} The in vitro release is also most commonly conducted in PBS 4.8 to mimic the release in lysosomal conditions. Lysosomes are membrane-enclosed organelles present in the cell, and function as the digestive system of the cell.¹⁸⁰ A great deal of in vitro release studies of LDHs have revealed that LDH release in PBS at pH 4.8 is sustained, but relatively faster compared to pH 7.4.^{22,23,37,39,41}

Kura et al intercalated the antiparkinsonian drug levodopa into LDHs and conducted in vitro release in PBS pH 7.4, wherein the drug release was found to be sustained until 8,500 minutes.¹⁷⁸ The release of levodopa in PBS solution of pH 4.8 was completed in 2,400 minutes; this release was still considered sustained, but was relatively faster than the release at pH 7.4. Figure 11 shows the release of levodopa from LDHs in PBS of pH 7.4 and pH 4.8.¹⁷⁸

The release at pH 7.4 is for the evaluation of the oral route PBS, with pH 6.8 being used to mimic the intestinal environment, and pH 4.8 is used as the simulator for a lysosomal environment. Rives et al comprehensively reviewed release studies on LDHs.^{7,8} Rojas et al studied the drug release from LDHs using ibuprofen as a model drug in an intestinal-simulated PBS solution of pH 6.8 and a gastric-simulated solution (HCl in 0.05 mol·L⁻¹ NaCl) of pH 1.2.¹⁷⁶ They found the release at pH 6.8 was relatively similar with PBS 4.8 lysosomal pH. However, release in the gastric environment at pH 1.2 was much faster, such that the complete release took only about 200 minutes.¹⁷⁶ The dissimilar pattern of drug release

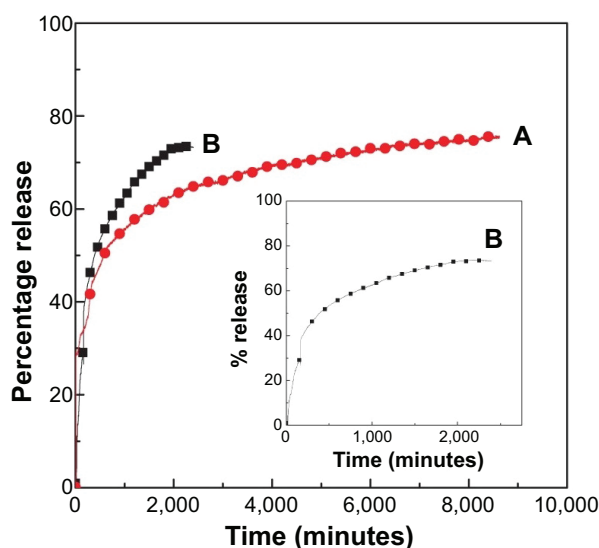


Figure 11 Release profiles of levodopa from the nanocomposite at pH 7.4 (A) and pH 4.8 (B).

Notes: Inset shows the release profiles of levodopa from the nanocomposite at pH 4.8 from 0 to 2,000 minutes. Reproduced with permission from Kura AU, Hussein Al Ali SH, Hussein MZ, Fakurazi S, Arulselvan P. Development of a controlled-release anti-parkinsonian nanodelivery system using levodopa as the active agent. *Int J Nanomedicine*. 2013;8:1103–1110.¹⁷⁸

from LDHs in unlike conditions is due to different release mechanisms. The drug can be released in two different ways: 1) an ion-exchange mechanism and 2) weathering mechanisms. The drug is released by an ion-exchange mechanism under basic or neutral PBS medium, whereas under acidic conditions the drug is released by both ion exchange and by the weathering of inorganic nanolayers.^{181,182}

Biocompatibility of layered double hydroxides

The most important characteristic of the ideal material for drug-delivery systems is biocompatibility with the human body, cells, and tissues. Biocompatibility can be evaluated by two means: in vitro and in vivo cytotoxic studies. In the in vitro studies, the compounds of interest are treated with different cell lines, and their viability is determined by different assays. Many cytotoxic studies are carried out over LDHs to evaluate their biocompatibility.

In vitro environment

Qin et al intercalated antitumor drug VP16 into LDHs and evaluated its antitumor effect, as well as its cytocompatibility with the human GES-1 cell line. They found that free VP16 was toxic to GES-1. On the other hand, LDH-intercalated VP16 did not show any cytotoxicity, and the therapeutic effect was maintained against the tumor cell lines MKN45 and SGC-7901.¹²⁸

Hussein Al Ali et al developed a formulation for the inhibition of angiotensin-converting enzyme by intercalating perindopril erbumine (PE) into LDHs. The study revealed that PE LDHs showed better enzyme-inhibition activity against angiotensin-converting enzyme, and the PE LDHs were found to be highly biocompatible with a human liver cell line.²⁴ Xu et al designed a gene-delivery system based on supercoiled pEF-eGFP plasmid and LDHs, and evaluated its cytocompatibility against HEK 293T cells. They found that pEF-eGFP LDHs were highly biocompatible with HEK 293T cells, even at the highest concentration of 500 µg/mL.¹⁷⁴ The protein gene LDHs have been reported to be biocompatible with kidney (Vero3) cells of monkeys by Masarudin et al, and Ramli et al reported ZnLH–salicylic acid biocompatibility with kidney (Vero3) cells of monkeys.^{183,184}

Marcato et al fabricated AgNP_{bio} with Mg–Al LDHs (LDH–AgNP_{bio}), and evaluated their biocompatibility against a permanent lung fibroblast cell line (V79).¹⁴⁶ The developed nanohybrid LDH–AgNP_{bio} was found to be highly biocompatible with the permanent lung fibroblast cell line (V79).¹⁴⁶ Saifullah et al developed an anti-TB nanocomposite formulation based on PAS and ZnLHs and Zn/AL LDHs. The cytotoxic study revealed that the anti-TB nanocomposites were highly biocompatible with human normal lung cells (MRC-5) and fibroblast cells (3T3).^{23,37} Barahuie et al designed an anticancer nanohybrid by intercalating PA into Mg/Al LDHs, and found the nanohybrid possessed strong anticancer activity against MCF-7 human breast cancer and human cervical cancer cell lines. At the same time, the nanohybrid did not show any cytotoxicity against fibroblast cells (3T3).¹²² Mohsin et al designed a sunscreen protection formulation that was able to cover the whole UV regions of A, B, and C by cointercalating the UV absorber CA, benzophenone-4, and Eusolex 232. The cytotoxic study of the UV-protection LDH formulation was found to be biocompatible with HDF cells.¹⁰¹ Hussein et al designed nanohybrid-based EA into ZnLHs and evaluated the cytotoxicity of the nanohybrid EA ZnLHs against the normal cell lines 3T3 and MCF-10A. The EA ZnLHs were found to be highly biocompatible with these normal cells, and at the same time EA ZnLHs were effective in killing two human cancer cell lines (breast cancer MCF-7 and liver cancer HepG2).¹⁸⁵

Kura et al studied the toxicity of a nanohybrid based on the antiparkinsonian drug levodopa and Zn/Al LDHs on the PC12 cell model. They found the nanohybrid did not induce any toxicity to the PC12 cells at a concentration of 100 µg/mL; however, levodopa in the free form was found to be extremely toxic, causing 80% cell death at 100 µg/mL.¹⁷⁸

In vivo environment

Clay minerals have been applied in pharmaceutical products. Hydrotalcite is used as an antacid and antipepsin agent under the brand names Almax, Bemolan, and Talcid. It is also used in cosmetic products, such as dentifrices and deodorants, as an excipient.^{186,187}

Indomethacin LDH in vivo biocompatibility

Indomethacin is an important anti-inflammatory drug applied for the treatment of ulcers, but it has gastrointestinal side effects. Del Arco et al intercalated indomethacin into Mg/Al LDHs and carried out a comparative pharmacological study of free indomethacin and Mg/Al LDH–indomethacin on Swiss mice of both sexes.¹⁸⁷ They found Mg/Al LDHs were able to reduce side effects and improve therapeutic outcomes against ulcers, and were much better compared to free indomethacin.¹⁸⁷

Ketoprofen LDH in vivo biocompatibility

Ketoprofen (Ket; 2-[3-benzoylphenyl]-propionic acid) is antipyretic, analgesic, and a nonsteroidal anti-inflammatory drug that is being applied for the cure of rheumatoid arthritis, osteoarthritis, and other chronic musculoskeletal conditions.^{188,189} Its application is limited by the side effects associated with it, such as peptic ulceration, anorexia, and bleeding.^{190,191} Sillion et al intercalated Ket into Mg/Al LDHs and Zn/Al LDHs and conducted an in vivo comparative biocompatibility study with free Ket in mice. Their study revealed that Ket LDHs reduced the ulcerogenic effect to a greater extent compared to free Ket.¹⁹²

Acetylsalicylic acid–dextran-coated LDH in vivo biocompatibility

Dong et al designed drug-delivery systems based on dextran-coated LDHs for acetylsalicylic acid, and conducted a pharmacokinetic study with rabbits as the model animal.¹⁶⁰ They found the LDH-based delivery system increased the bioavailability and half-life and reduced the side effects of acetylsalicylic acid.¹⁶⁰ Table 2 summarizes the in vitro cytotoxic studies of inorganic nanolayers.

LDH nanoparticle in vivo biocompatibility

Yu et al evaluated acute oral toxicity and kinetic behaviors of LDH NPs by using mice as the model animal. They found that LDH NPs did not cause any abnormal behaviors, mortality, symptoms, body-weight loss, or any symptoms at the very high concentration of 2,000 mg/kg for 14 days. Furthermore, their study revealed that LDH NPs did not cause any injury to the liver or kidneys.¹⁹³ Flesken-Nikitin et al evaluated the

toxicity of Mg/Al LDH NPs and used different routes in mice, namely subcutaneous, intraperitoneal, and intravenous injections. The LDH NPs showed only minor *in vivo* toxicity, and their results were highly encouraging, with a high percentage of mouse survival.¹⁹⁴

Kura et al recently evaluated the subacute oral toxicity of levodopa–Zn/Al LDHs in rats of 5–500 mg/kg body weight. The study revealed that there was no significant change in body-weight gain, water intake, or percentage of survival between groups of rats treated with nanocomposites and the control group. In addition, the liver, spleen, and brain histology was similar with the control group.¹⁹⁵

In vivo biocompatibility is the most important characteristic of any material considered for biomedical application. The aforementioned *in vivo* biocompatibility studies are highly encouraging, but there is still need for further *in vivo* studies on LDHs in order to establish that they are safe for biomedical applications. Table 3 summarizes the *in vivo* cytotoxic studies of Inorganic nanolayers.

Conclusion

There are varieties of materials being studied for biomedical and other applications. However, inorganic nanolayers are unique and very useful in many aspects, such as their easy preparation and proven *in vitro* and *in vivo* biocompatibility. Their tendency to intercalate a variety of anions, such as inorganic and organic acids, organic dyes, pharmaceutical drugs, and biopolymers, and being able to be fabricated with other metallic materials like silver and ceramics either by intercalation or by surface interactions, resulting in fabulous material with numerous applications, makes them all the more advantageous.

The development of drug-delivery science is an important avenue in modern biomedical research. Inorganic nanolayers have been continuously exploited for the delivery of a number of drugs, and have been found to be the best material to be applied as a delivery system. For a material to be suitable for drug delivery, it should meet some important criteria: 1) it should be biocompatible with normal cells and tissues, 2) have a tendency to encapsulate/intercalate different pharmaceutical drugs, 3) be easy to prepare, 4) be cost-effective, 5) be able to release the drug in a sustained manner, 6) be able to deliver the drug at the disease site, and 7) be biodegradable and easily excreted from the body after releasing the drugs. This review has categorically proved the ability of nanolayers to meet these criteria, and hence these are an ideally suitable biomaterial to be applied for drug-delivery purposes.

Gene therapy is another revolutionary modern technique of biomedical science in which defective genes are replaced

by healthy genes. Previously, viruses were being employed as gene carriers, but due to the risk of adverse health effects, scientists are looking for nonviral gene carriers. Inorganic nanolayers have been applied as nonviral gene carriers recently in gene delivery. Our review has shown that inorganic nanolayers have successfully transfected genes. Their positive surface charge assists them to cross the negatively charged cell membrane. Gene delivery is also one of the most important characteristics of nanolayers along with drug delivery.

The diagnosis of disease is another important research area of modern biomedical science. Previously, people needed to submit samples from the human body (such as blood, urine, and bone marrow) and wait for hours to days to get the reports. In modern medical diagnosis, a shorter time to reach diagnosis can be the difference between life and death. Biosensing technology has made it possible to diagnose diseases in a matter of seconds. Most importantly, a layman can easily understand the output of the results by eliminating the need of a laboratory expert to interpret the results.

Recently, inorganic nanolayers have been applied in biosensors, and are considered to be a suitable material, as they can easily intercalate the biosensing receptor biomolecules, are highly biocompatible, and provide a high surface area to facilitate faster electron transfer during biosensing activity. The application in the biosensors enhances the versatility of the inorganic nanolayers and further widens horizons for biomedical science. Inorganic nanolayers have the tendency to perform dual functions as a biosensor and drug-delivery system. This simultaneous dual-function characteristic has made it possible to identify the disease site and deliver the drug there. This has opened up a new research dimension in biomedical sciences.

The diagnosis of disease and therapy is collectively called theranostic science, and this has been facilitated by the use of inorganic nanolayers, which are fabulous biomaterials applied in theranostic science.

Bioimaging is also an important area of biomedical science that can be applied in the diagnosis of disease, monitoring the disease condition, and applying to the study of normal human physiology and anatomy. Most recently, inorganic nanolayers have been applied in bioimaging science, and results suggest that there are many fascinating biomaterial applications to be applied in bioimaging science. Inorganic nanolayers are suitable for bioimaging science, because of their proven high biocompatibility, ability to be intercalated with fluorescent organic material that can glow, and ability to be fabricated with metals that can confer the

glow to them. Furthermore, the positively charged surface of the inorganic nanolayers would assist in internalization inside the cell. Only a few studies have been carried out recently on bioimaging applications of inorganic nanolayers. The initial results of these are highly encouraging.

The application of inorganic nanolayers in biosensors, drug delivery, gene delivery, and bioimaging biomaterial will enable us to diagnose the disease, release the drug at the target site, and help in monitoring therapeutic progress. It can be concluded that inorganic nanolayers are an excellent biomaterial for various biomedical applications, such as cosmetics, drug delivery, biosensing, and bioimaging technology.

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Disclosure

The authors report no conflicts of interest in this work.

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