Controlled synthesis of layered double hydroxide compounds with novel morphologies for biological applications

Poernomo Gunawan

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**CONTROLLED SYNTHESIS OF LAYERED DOUBLE
HYDROXIDE COMPOUNDS WITH NOVEL
MORPHOLOGIES FOR BIOLOGICAL APPLICATIONS**

**POERNOMO GUNAWAN
SCHOOL OF CHEMICAL AND BIOMEDICAL ENGINEERING
2009**

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HYDROXIDE COMPOUNDS WITH NOVEL MORPHOLOGIES
FOR BIOLOGICAL APPLICATIONS

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School of Chemical and Biomedical Engineering

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Summary

The research work presented in this thesis focuses on the synthesis and characterization of drug containing layered double hydroxides (LDHs), a group of anionic clays, with novel properties. Owing to their flexibility in accommodating anionic compounds in their interlayer galleries, they are potential for developing materials with a wide range of applications. In particular, LDH intercalated with organic anions, named as organo-LDH, is one of the most important organic-inorganic hybrid materials that have recently attracted considerable attention in pharmaceutical field.

The main research work is to prepare drug-containing LDHs with novel structures and properties. First, we present the work on producing ibuprofen intercalated LDH nanocrystals with different particle size and morphology under different synthesis conditions including solvent type, and aging conditions in chapter 4. As a result, they further affect inter particle interactions that give different aggregation states in the dry powders. It was found that the relatively oriented dense powder through preferential face-to-face and edge-to-edge aggregation of LDH nanoparticels results in a considerably slower release rate of ibuprofen compared with that from the loose ones.

Self-assembly of LDH nanoparticles to form coral-like morphology using a solvothermal method in a nonaqueous polar solvent/surfactant system of EG (ethylene glycol)/methanol/DS (dodecyl sulfate) was achieved and is presented in chapter 5. A detailed study on the effects of solvothermal reaction temperature, duration and solvent type has revealed the formation of an intermediate compact microsphere structure via self-assembly of LDH nanoparticles. Through recrystallization, it further evolves to form the coral-like porous structure with nanoplatelets as the building unit. The dodecyl sulfate anions intercalated in the pristine coral-like LDHs was then readily exchanged by drug anions while the morphological features were well maintained.

Chapter 6 presents the fabrication of hollow LDH nanospheres using carbon nanospheres (CNS) as templates. It demonstrates direct assembly of the preformed anisotropic LDH nanocrystals (30×10 nm) on the surface of CNS (~ 800 nm) in one step. Closely packed LDH films were formed on the CNS template. After the carbon core removal via calcination, hollow nanospheres of MgAl-oxides with robust shell walls are formed. The oxide shell can be readily converted to LDHs intercalated with functional anions (e.g., drug anions) based on the well-known memory effect.

Introduction of other functionality into LDHs is presented in chapter 7. Tb^{3+} cation was successfully incorporated in the lattice of the hydroxide layers to impart photoluminescence property. A systematic study on the effects of Tb^{3+} amount and the type of interlayer anions on the luminescence efficiency was conducted. It has also been found that certain types of interlayer drug anions, which contain cyclic structure or benzene rings, also act as sensitizers for efficient absorption and transfer of energy to Tb^{3+} , thus giving rise to enhanced photoluminescence signals at longer wavelengths. The compounds developed in this work have a greater flexibility in tuning the amount of rare earth element, varying the type of interlayer anions for specific applications and extending the excitation wavelength by choosing appropriate interlayer anions as sensitizers.

Nomenclature

δ	bending vibration mode in infrared spectrum
ζ	zeta potential
θ	diffraction angle in X-ray diffraction measurement ($^{\circ}$)
λ	wavelength
μA	micro-Ampere
ν_{as}	asymmetric stretching vibration mode in infrared spectrum
ν_{n}	symbol of infrared vibrational mode
ν_{s}	symmetric stretching vibration mode in infrared spectrum
τ	luminescence lifetime
a	unit cell in parameter LDH (inter-cation distance in brucite-like layer)
AES	atomic emission spectroscopy
AOT	sodium bis(2-ethylhexyl) sulfosuccinate
As- <i>myc</i>	c-antisense oligonucleotide
ATP	adenosine triphosphate
BDC	2,2'-bipyridine-5,5'-dicarboxylate
BE	binding energy
BET	Brunauer-Emmet-Teller
BJH	Barrett-Joyner-Halenda
BPA	4-biphenylacetic acid
c'	distance of two brucite-like layer
c	$3c'$ or $2c'$ (unit cell parameter of LDH in c -direction: rhombohedral or hexagonal, respectively)
C	co-precipitation method
CHN	carbon-hydrogen-nitrogen
CMC	critical micelle concentration
CNS	carbon nanosphere
d	distance between two crystal planes
D_p	crystallite size
DBS	dodecylbenzene sulfonate
DNA	deoxyribo nucleic acid
DS	dodecyl sulfate
DTA	differential thermal analysis
DTPA	diethyl triamine pentaacetate

EDX	energy dispersive X-ray spectroscopy
EELS	electron energy loss spectroscopy
EG	ethylene glycol
EM	emission
eV	electronVolt
EXC	excitation
FA	folic acid
FTIR	Fourier transformed infrared spectroscopy
FU	5-fluorouracil
FWHM	full width at half maximum
h	hour
H	hydrothermal method
HTlc	hydrotalcite-like compound
<i>I</i>	intensity
Ibp	ibuprofen
ICP	inductively coupled plasma
kV	kiloVolt
LBL	layer-by-layer
LDH	layered double hydroxide
m	mole of water content in the interlayer
M ^{II}	divalent cation
M ^{III}	trivalent cation
M ^{IV}	tetravalent cation
M	molarity of solution (concentration unit)
MA	methanol
mL	mililiter
MTX	methotrexate (anticancer drug)
<i>n</i>	refractive index
Nap	naproxen
NCs	nanocrystals
NSAID	non-steroidal anti-inflammatory drug
<i>OD</i>	optical density
PAH	polyallylamine hydrochloride
PBS	phosphate buffer saline
PEG	poly(ethylene glycol)

P_o	Poisson noise
PSS	poly(4-styrene sulfonate)
Q	quantum yield
SDS	sodium dodecyl sulfate
sec	seconds
SEM	scanning electron microscopy
STEM	scanning transmission electron microscope
TCD	thermal conductivity detector
TEM	transmission electron microscopy
TGA	thermogravimetric analysis
UV	ultraviolet spectroscopy
VA	valproic acid
W	water
x	molar ratio of $M^{II} : (M^{II} + M^{III})$
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

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Publications Related to the Thesis

Journal papers

1. Gunawan, P. and Xu, R., Lanthanide-doped Layered Double Hydroxides Intercalated with Sensitizing Anions: Efficient Energy Transfer between Host and Guest Layers, *Journal of Physical Chemistry C*, 113, 17206-17214, 2009.
2. Gunawan, P. and Xu, R., Direct Assembly of Anisotropic Layered Double Hydroxide (LDH) Nanocrystals on Spherical Template for Fabrication of Drug-LDH Hollow Nanospheres, *Chemistry of Materials*, 21, 781-783, 2009.
3. Gunawan, P. and Xu, R., Synthesis of Unusual Coral-like Layered Double Hydroxide Microspheres in a Nonaqueous Polar Solvent/Surfactant System, *Journal of Materials Chemistry*, 18, 2112-2120, 2008.
4. Gunawan, P. and Xu, R., Direct Control of Drug Release Behavior from Layered Double Hydroxides through Particle Interactions in Solid Aggregates, *Journal of Pharmaceutical Sciences*, 97, 4367-4378, 2008.

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5. Gunawan, P., and Xu, R. Development of nanostructured layered double hydroxides for potential biomedical applications, The International Symposium on Advanced Bio, Nano, and Pharmaceutical Science and Technology, May 2009, Beijing, China.
6. Gunawan, P., Chen, F. X. and Xu, R. Synthesis of Novel Organo-Layered Double Hydroxide and Thermal Conversion to Nanocomposite Materials, Regional Symposium on Chemical Engineering, 4-6 December 2007, Yogyakarta, Indonesia.
7. Gunawan, P. and Xu, R. Synthesis of Novel Hydrotalcite-like Compounds for Biological and Pharmaceutical Applications, International Conference on Exploring the Frontier of Nano, Bio, and Pharmaceutical Technology, 26-28 September 2006, Tianjin, People's Republic of China.

CHAPTER 1 INTRODUCTION AND THE SCOPE OF THESIS

The research work presented in this thesis is focused on the synthesis and characterization of drug containing layered double hydroxides (LDHs) with novel properties. The main objective is to develop this type of materials for potential applications in pharmaceutical field.

Layered double hydroxide, also well known as hydrotalcite-like compound (HTlc), is a general term for a large group of anionic clays. As indicated in their name, they are layer-structured materials in which the hydroxide layer consists of two or more different kinds of cations with different valences. In the current work, Mg^{2+} and Al^{3+} are used as cations in all the materials syntheses due to their biocompatibility; hence it is abbreviated MgAl-LDHs. In MgAl-LDHs, Al^{3+} cation partially substitutes Mg^{2+} cation in the octahedron formed by the OH^- groups. Thus, the hydroxide layers of LDHs are positively charged. To balance the net positive charge, exchangeable anions are intercalated into the interlayer space, and at the same time, water molecules are also incorporated into the expanded interlayer space. The composition of LDHs can be represented by a general formula, $[M^{II}_{1-x}M^{III}_x(OH)_2]^{x+}A^{n-}_{x/n} \cdot mH_2O$ (M = metal cation, A = interlayer anion). Large varieties of anions ranging from simple inorganic ones such as CO_3^{2-} , NO_3^- , OH^- , Cl^- , SO_4^{2-} , etc., to complex compounds, such as polyoxometalates, polymeric anions and even DNA anions can be incorporated in the interlayer gallery of LDHs. Hence, it presents an enormous potentiality for preparing materials with a wide range of applications. In particular, LDHs intercalated with organic anions, named as organo-LDHs, as one of the most important guest-host materials have recently attracted considerable attention in pharmaceutical field.

Recently, LDHs have been explored for drug delivery and controlled drug release, as well as for elimination of the negative side effects of the drugs.¹⁻⁶ Owing to their simplicity in synthesis, biocompatibility, low toxicity, and controllable solubility, LDHs are potential candidates towards such applications. In order to facilitate efficient and targeted delivery of

bioactive molecules, many tunable properties of organo-LDHs need to be investigated and controlled according to pharmacological needs. To achieve success in such applications, the particle sizes, structural features, and other functional properties of the materials need to be carefully designed. The amount of intercalated bioactive molecules, their orientation, and interaction with the host layers are also important factors.

In the current PhD research program, the main work conducted is summarized as follows:

- 1) Synthesis of MgAl-LDHs containing ibuprofen, a model drug, with various morphologies and different particle sizes by varying the solvent system. Co-precipitation followed by either aging at ambient conditions or hydrothermal treatment was employed to produce the desirable particle-particle interaction of dried drug-LDH nanocompounds. As a result, the drug release rate from the dry powders was controlled.
- 2) Synthesis of unique coral-like MgAl-LDH structures in a mixture of nonaqueous polar solvent/surfactant system of EG (ethylene glycol)/methanol/DS (dodecyl sulfate) via a one-pot synthesis method under solvothermal conditions.
- 3) Synthesis of hollow nanospheres of MgAl-LDHs using carbon nanospheres (CNS) as templates through direct self-assembly of colloidal LDH nanocrystals on the CNS surface via electrostatic interactions.
- 4) Incorporation of rare earth element in the hydroxide layers for the fabrication of luminescent LDHs with sensitising agent in the interlayer spacing to enhance fluorescence intensity via energy transfer.

Following this introduction, Chapter 2 provides an overview on LDHs with regards to their physicochemical properties, synthesis methods, and the recent development of LDHs for pharmaceutical applications. In addition, a brief review on other materials as drug carriers is also discussed for comparison purpose.

The general experimental methodologies and the principles of materials characterization techniques used throughout the research work are briefly presented in Chapter 3.

Chapter 4 presents the work on producing ibuprofen intercalated LDH nanocrystals with different particle size and morphology under different synthesis conditions including solvent type and aging conditions. It has been found that different synthesis conditions impinge on different crystallization rates. As a result, the crystallinity, the particle size, and the size distribution are affected. These properties further affect particle-particle interactions during the assembly of particles upon drying. Different aggregation states in the dry powders of ibuprofen intercalated MgAl-LDHs can be obtained. The effect of aggregation states of LDH nanocrystals on the drug release behavior was studied. It was found that the relatively oriented dense powder through preferential face-to-face and edge-to-edge aggregation of LDH nanoparticels gave rise to a considerably slower release rate of ibuprofen compared with that from the loose ones. This can be attributed to a less breakdown of aggregates, a longer diffusion path length, and a higher diffusion resistance in the oriented solid matrix.

The commonly occurred crystallite morphology of LDHs is anisotropic platelets. They form irregular aggregates upon natural drying, which may affect their performance in many applications. Chapters 5 and 6 present our efforts in fabrication of LDHs with well-defined structures. Chapter 5 demonstrates the synthesis of coral-like LDHs using a solvothermal method in a nonaqueous polar solvent/surfactant system of EG (ethylene glycol)/methanol/DS (dodecyl sulfate). The mechanism of forming such a unique LDH structure was investigated. The detailed study on the effects of solvothermal reaction temperature, duration, and solvent type has revealed the formation of an intermediate compact microsphere structure via self-assembly of LDH nanoparticles. Through recrystallization, it further evolves to form the coral-like porous structure with nanoplatelets as the building unit. The dodecyl sulfate anions intercalated in the pristine coral-like LDHs was then readily exchanged by drug anions while the morphological features were well maintained.

Chapter 6 presents the fabrication of hollow LDH nanospheres using carbon nanospheres (CNS) as templates. It has been demonstrated that direct assembly of the preformed anisotropic LDH nanocrystals (30×10 nm) on the surface of CNS (~ 800 nm) can be achieved in one step. Closely packed LDH films were formed on the CNS template. After removing the core via calcination, hollow nanospheres of MgAl-oxides with robust shell walls are formed. The oxide shell can be readily converted to LDHs intercalated with functional anions (e.g., drug anions) based on the well-known memory effect. With the hollow structure, it is possible that other functional molecules or nanoparticles can be incorporated into the interior space to attain multifunctional composite nanospheres.

Besides functioning as drug carriers, LDHs have also been developed into potential candidates for bioimaging. Chapter 7 presents the synthesis of luminescent LDHs by incorporating a rare earth element, Tb^{3+} cation, in the hydroxide layer. A systematic study on the effects of Tb^{3+} amount and the type of interlayer anions on the luminescence efficiency was conducted. Interestingly, it has been found that certain types of interlayer drug anions which contain cyclic structure or benzene rings act as sensitizers for efficient absorption and transfer of energy to Tb^{3+} , thus giving rise to enhanced photoluminescence signals at longer wavelengths. The stability of LDH layers in confining Tb^{3+} ions was investigated to assess its feasibility as drug carriers and at the same time as photoluminescent materials. The leaching study reveals that Tb^{3+} cations are stably contained in the host LDH lattice for a long period in the neutral environment. Different from the rare earth complex intercalated LDHs, the compounds developed in this work have a greater flexibility in tuning the amount of rare earth element, varying the type of interlayer anions for specific applications and extending the excitation wavelength by choosing appropriate interlayer anions as sensitizers.

Lastly, Chapter 8 summarizes the major results and findings of the proceeding chapters. Outlook and suggestions for future work are provided at the end of the chapter.

CHAPTER 2 LITERATURE REVIEW

2.1 Materials Developments as Carriers of Bioactive Molecules

Throughout the past decades, developments of materials as drug carriers both in micron and submicron size have been significantly pursued in the search of biocompatible and effective carriers with desired therapeutic properties. Both organic-based materials and inorganic-organic hybrids have been explored extensively for such purposes. Among these systems, polymeric-based carriers have been most widely investigated. A broad range of polymers has been applied in the pharmaceutical field which has been well reviewed.⁷⁻¹⁰ In general, both hydrophobic and hydrophilic polymers can be used as drug carriers, but suitable for different applications.¹¹ Hydrophobic polymers have advantages in stronger interaction with drugs and cell membranes due to hydrophobicity of the membranes. Hydrophobic polymers studied so far include polylactide-co-glycolide which is biodegradable,¹² poly- ϵ -caprolactone, polyhydroxyalkanoates,¹³⁻¹⁴ polyorthoesters,¹⁵ polyphosphoesters,¹⁶ polyanhydrides,¹⁷⁻¹⁸ etc. On the other hand, hydrophilic polymers are bioadhesive (mucoadhesive) owing to their ability to form hydrogen bonding and chain entanglement. Hence, this gives benefits in localizing drug concentration at the surface of gastrointestinal tract for oral administration and minimizing diffusional distance for drug release from polymer matrix. The frequently studied hydrophilic polymers include polyacrylates,¹⁹ polyalkylmethacrylates, polyethylene glycol,²⁰⁻²¹ and hydrogels.²²⁻²⁴ Polyalkylmethacrylates are usually used as enteric coating agents, as their dissolution is triggered by the changing of pH.²⁵ They are not soluble in acidic medium but soluble in neutral to alkaline environment. Together with polyalkylmethacrylates, cellulose acetate phthalate, hydroxypropyl methyl cellulose phthalate are also widely used for controlling drug release in gastrointestinal tract.²⁶ Meanwhile, polyethylene glycol is commonly used to coat the surface of nanoparticles to

prolong the circulation time of particles in the blood course, thus increasing the chance of drug delivery to the targeted site.^{21, 27-28}

In addition to polymers, other carrier types that have been extensively developed are viral carriers,²⁹ polyelectrolites,³⁰⁻³³ recombinant proteins,³⁴ and inorganic nanoparticles such as magnetite,³⁵ gold,³⁶ calcium phosphates,³⁷ mesoporous silica³⁸⁻³⁹ porous silicon,^{37, 40} and layered double hydroxides.⁴¹⁻⁴⁴ The widely employed delivery systems for DNA include viral vectors and recombinant proteins due to their low immunogeneity. In viral carriers, certain part of the viral original gene segment is eliminated to allow space for foreign genes to be inserted and delivered. Meanwhile, recombinant proteins can mimic different types of viral properties via integrating various peptide segments that are required for efficient gene transfer through protein engineering.²⁹ By a layer-by-layer (LBL) technique using both polyallylamine hydrochloride (PAH) and poly-4-styrene sulfonate (PSS), hollow capsules were formed for loading of bioactive molecules, such as anticancer doxorubicin,³¹ dextran, or albumin.³³ The slow degradation of polyions also enables the control of the drug release rate.³⁰⁻³¹ In addition, other polycationic compounds, such as polyethyleneimine and poly(L-lysine) have also been widely used as non-viral delivery agents.^{32, 45}

As novel drug delivery systems, inorganic nanoparticles offer versatile properties that have attracted much attention recently. Magnetic nanoparticles, such as iron oxide^{35, 46} and cobalt,⁴⁷ as well as gold nanoparticles³⁶ can be easily synthesized with different sizes. Moreover, they are also easily conjugated with other biomolecules or polymers as linkers to immobilize drug compounds or gene moiety on their particle surfaces. In addition, mesoporous silica, such as MCM-41 and SBA-15, are potential drug carriers due to their porous structures with large pore volumes and pore sizes that enable absorption or encapsulation of relatively large amounts of bioactive molecules.³⁹ Several reports demonstrated that silica can be used to store and gradually control the release of drugs like antibiotics⁴⁸ and camptothecin.⁴⁹ Furthermore, due to its biocompatibility, silica is also used

to promote the biocompatibility of some drug delivery systems. Lai et al. reported the inclusion of quantum dots inside silica mesopores together with drug molecules.⁵⁰ Deposition of thin layer of silica onto nanoparticles is reported by other groups, such as the coating of magnetic nanoparticles,⁴⁶ the formation of core/shell gelatine/silica nanoparticles,⁵¹ and the cross-linking of silica and micellar nanoparticles.⁵²

Similar to mesoporous silica, porous silicon also demonstrates good biocompatibility. With porosity that may reach 80%, porous Si is able to carry various biologically active compounds such as proteins, enzymes, and drugs with high loading capacity.⁴⁰ *In vitro* release of the steroid dexamethasone,⁵³ doxorubicin,⁵⁴ and many other oral administered drugs of poor solubility, such as furosemide, ibuprofen, ranitidine, and antipyrine, has been conducted using the porous Si as the carrier.⁵⁵ The release rate of dexamethasone in phosphate buffer saline (PBS) was linear up to 50% of loaded amount within the initial 2 h followed with slow release rate up to 3 days.⁵³ The release study of doxorubicin using human colon adenocarcinoma cell lines LoVo showed a release profile that reached a plateau within 5 h.⁵⁴ Salonen, et al. reported the loading of low soluble drugs in the gastrointestinal track and conducted *in vitro* drug release in media with different pH at 5.5, 6.8, and 7.4. The study at pH 7.4 showed that 80% release of antipyrine, ranitidine, ibuprofen, and furosemide were obtained within 75, 59, 35, and 41 min, respectively.⁵⁵ Based on these studies, it is apparent that porous silicon can be potentially used as drug delivery agent.

Besides establishing numerous materials systems as drug carriers, surface modifications and materials engineering are essential to control their biological properties in a desired fashion in order to generate a new generation of carriers that are able to simultaneously perform various therapeutic and diagnostic functions.⁵⁶ One may desire a smart drug carrier that possesses the following properties: (1) specifically targeted to a diseased organ to enhance patient compliance, (2) bearing a good contrast for real-time observation, (3) long circulation in blood course, (4) responsive to local stimuli, such as pH or

temperature changes to control the drug release. In the light of this direction, new developments in combining several functionalities in a single carrier are required, as illustrated in Figure 2-1.

Figure 2-1. A schematic picture of a multifunctional drug carrier: (1) a conventional drug-loaded nanoparticle without any other functionality; (2) drug-loaded nanoparticle containing magnetic particles; (3) polymer-grafted drug-loaded nanoparticle (e.g. PEG) for prolonged circulation of nanocarriers in the blood course; (4) drug-loaded nanoparticle functionalized with a specific ligand on its surface, such as antibody or folic acid, for targeted delivery; (5) photoluminescent drug-loaded nanoparticle for imaging and particle tracing; (6) drug-loaded nanoparticle containing cell-penetrating peptide such as CPP attached to the surface to enhance cell uptake; (7) illustrated multifunctional pharmaceutical nanoparticle with combined properties. Reproduced from [56]. Copyright 2006, with permission from Elsevier.

A number of studies have been endeavored to prepare nanoparticles with multifunctionality. Such examples are the synthesis of drug-loaded chitosan nanoparticles with magnetic and fluorescence properties,⁵⁷ the binding of antibody as targeting ligand onto rare earth complexes to render targeted imaging and therapy,⁵⁸ and the functionalization of doxorubicin-loaded magnetite nanoparticles with PEG as stealthing agent and folate moiety as receptor.⁵⁹ With these successful attempts, further development of smart materials even with

more complex characteristics can be possibly achieved, hence driving the frontier in biomedical research to another height.

Layered double hydroxides (LDHs), a class of anionic clay, have lately emerged as a type of promising inorganic carriers. Such materials are the main focus of this thesis. Their versatility in accommodating various anionic molecules and high biocompatibility are some of great advantages that the materials offer. Detailed explanations on the physicochemical properties and the advancement of LDHs as drug carriers are discussed in the remaining part of this chapter.

2.2 Introduction on Layered Double Hydroxides

Clays are commonly used natural minerals which have wide applications in many fields. Clays are frequently used as building materials, foundry moulds, paper coatings and fillings, drilling mud, and even pharmaceuticals, etc. Furthermore, clays have been applied as adsorption materials and catalytic materials, etc., depending on their compositions and specific properties. These wide-field applications of clays are largely due to their high surface area, large porosity, great intercalation ability, and high water retention capacity.

In general, clays can be classified into two broad categories: cationic and anionic clays. Cationic clays possess negatively charged alumino-silicate layers, thus positively charged compounds incorporated in the interlayer space will balance the charges. On the other hand, anionic clays consist of positively charged metal hydroxide layers that lead to the intercalation of anionic compounds between the layers to balance the positive charges.

Layered double hydroxide (LDH) is well known as one important class of anionic clays. It is also known as hydrotalcite-like material, consisting of layers that contain hydroxides of metal cations with different valences. So far, this type of materials has been the subject of numerous scientific publications due to their wide applications. However, their

application as pharmaceutical carriers have been studied recently and it shows great potentialities.

2.2.1 Structural Properties of Layered Double Hydroxides (LDHs)

The layered structure of LDHs is closely related to that of brucite, $\text{Mg}(\text{OH})_2$ ⁶⁰ as shown in Figure 2.2a. In brucite layer, each Mg^{2+} ion is surrounded by six hydroxide ions in octahedral arrangement. Through sharing edges, the octahedron forms two-dimensional sheets that are stacked one on top of another by weak interactions due to hydrogen bonding. When Mg^{2+} ions are partially replaced by Al^{3+} ions, it results in the layers possessing a net positive charge. This replacement together with the intercalation of charge-balancing carbonate anions in the interlayer space gives rise to a new material, so-called hydrotalcite with a formula of $\text{Mg}_6\text{Al}_2(\text{OH})_{16}\text{CO}_3 \cdot 4\text{H}_2\text{O}$ ⁶⁰ (Figure 2.2b and c). Due to the expansion of the interlayer space, some water molecules are also incorporated.

Figure 2-2. Schematic diagrams of (a) brucite lattice; (b) hydrotalcite lattice; and (c) atom composition.

The brucite-like sheets of LDHs may have two stacking sequences, rhombohedral (3R symmetry) and hexagonal (2H symmetry)⁶¹, which can be distinguished by crystallographic analysis. Normally, synthetic hydrotalcite adopts a rhombohedral 3R stacking sequence. The rhombohedral-type hydrotalcite has lattice parameters of a ($= b$), which is the distance between two adjacent cations, and c ($= 3c'$), where c' is the sum of thickness of one brucite-like layer and one interlayer (gallery). On the other hand, the hexagonal polytype form of

hydrotalcite, manasseite, has parameters of $a = b$ and $c = 2c'$. The hexagonal type can be obtained by thermal transformation of the rhombohedral form.⁶¹

The properties of LDH materials are mainly determined by the composition of the hydroxide layers, the type of interlayer anions and their position and orientation, and the water content in the interlayer region. These aspects will be discussed separately in the following subsections.

2.2.2 Metal composition of hydroxide layers

The well-known compositional flexibility of LDHs is demonstrated by a wide range of cations as well as interlayer anions which can be accommodated in the LDH structure. In general, a formula $[M^{II}_{1-x}M^{III}_x(OH)_2]^{x+}(A^{n-x/n}) \cdot mH_2O$ ⁶² (where M = metal, A = interlayer anion) can be used to represent most of these compounds. The formula describes the most commonly encountered LDHs formed by divalent and trivalent cations which have ionic radii similar to that of Mg^{2+} , such as Mg^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , and Al^{3+} , Cr^{3+} , Mn^{3+} , Fe^{3+} , Co^{3+} , etc. It is also common to synthesize LDHs with more than one divalent cation and/or more than one trivalent cation in the hydroxide layers. In addition, although less commonly encountered, another two types of LDHs can be readily synthesized with the options of (i) M^I - M^{III} cation pairs, and (ii) M^{II} - M^{IV} cation pairs or M^{II} - M^{III} - M^{IV} cation combinations. Some common examples of the cation combinations found in literatures are Li^+ - Al^{3+} ,⁶³⁻⁶⁴ Co^{2+} - Ti^{4+} , Mg^{2+} - Al^{3+} - Zr^{4+} , and Co^{2+} (or Ni^{2+})- Al^{3+} - Sn^{4+} .⁶⁵⁻⁶⁷

Due to space confinement, to successfully form LDHs, the ionic radii of both divalent and trivalent cations should not be too different from that of Mg^{2+} ($\leq 15\%$) so that they can be suitably accommodated in octahedral lattice by closely packed $-OH$ groups. Ionic radii of some common divalent and trivalent cations in the octahedral coordination are listed in Table 2.1. Many of these cations have reasonably similar radii as that of Mg^{2+} , except for Be^{2+} , Ca^{2+} and Cd^{2+} , which are either too small or too big and thus not suitable to form LDHs.

However, the formation of LDHs incorporated with a small amount of Ca^{2+} inside the hydroxide layers was reported occasionally.⁶⁸

Table 2-1. Ionic radii of some divalent and trivalent cations.

Divalent (M^{II})		Trivalent (M^{III})	
Cation	Radius (Å)	Cation	Radius (Å)
Be	0.30	Al	0.50
Mg	0.65	Ga	0.62
Cu	0.69	Ni	0.62
Ni	0.72	Co	0.63
Co	0.74	Fe	0.64
Zn	0.74	Mn	0.66
Fe	0.76	Cr	0.69
Mn	0.80	In	0.81
Ca	0.98	Ti	0.76

Besides the type of metal cations, the ratio of divalent to trivalent cations is another factor for the successful synthesis of pure LDHs. It is generally agreed that LDHs can be formed with x value (where $\text{M}^{\text{II}}/\text{M}^{\text{III}} = (1-x)/x$) between 0.1 and 0.5. However, many studies have indicated that pure phases only exist for $0.2 \leq x \leq 0.33$; otherwise, different compounds will be obtained. For Mg-Al system, low number of Al ($x < 0.2$) may lead to the formation of $\text{Mg}(\text{OH})_2$ segregation due to the high density of Mg in the brucite-type sheets. Analogously, high number of Al will instead produce $\text{Al}(\text{OH})_3$ phase, which is gibbsite. These side phases are amorphous in many cases; therefore, they are not detectable by the common crystallographic technique.⁶¹

2.2.3 Nature of interlayer anions

As the interlayer anions function as balancing agents for the positively charged hydroxide layers, there is essentially no limitation to the type of anions to be incorporated in LDHs provided that the anions do not destruct the structure of metal hydroxide layers. The only problem related with the sample preparation is that it is difficult to get pure and well-crystallized LDH without carbonate contamination when anions different from carbonate are intended to be intercalated. This is because the presence of CO_2 in the environment is unavoidable and it has high affinity to brucite-like layers. In fact, the easiness of anion intercalation is dependent on the affinity of the anions. Among the commonly intercalated inorganic anions, the order is $\text{CO}_3^{2-} > \text{HPO}_4^{2-} > \text{CrO}_4^{2-} > \text{SO}_4^{2-} > \text{OH}^- > \text{F}^- > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{I}^-$. So far, many studies regarding intercalation of different anions can be found in the literature:

- 1) common inorganic anions like carbonate,⁶⁹⁻⁷⁵ nitrate,⁷⁶⁻⁷⁷ chloride,⁷⁸⁻⁷⁹ sulphate,^{73, 80} chromate,⁸¹⁻⁸² molybdate,⁸³ phosphate, hydrogenophosphate;^{78, 84}
- 2) organic compounds like carboxylic acid,⁸⁵⁻⁸⁶ aliphatic and aromatic dicarboxylate,⁸⁷⁻⁸⁸ sulfonate,⁸⁹ phosphonate;⁹⁰
- 3) complex anions like hexacyanoferrate,⁹¹⁻⁹⁵ polyoxometalate;⁹⁶
- 4) polymeric anions like poly(styrene sulfonate), and poly(vinyl sulfonate),⁹⁷ polyethylene,⁹⁸ polymethylmetacrylate;⁹⁹
- 5) biochemical compounds like anionic drugs,^{4-5, 100-101} ATP,¹⁰² sugar,¹⁰³ amino acids,¹⁰⁴⁻¹⁰⁷ nucleotide and DNA;³
- 6) various kinds of anionic surfactants.¹⁰⁸⁻¹¹¹

Anions of different structures, dimensions, and charges give rise to different orientation, packing, and interactions with the hydroxide layers, which determine the interlayer distance, i.e. the c parameter.

In general, the anions arrange themselves in the interlayer in such a way as to give maximum electrostatic interactions with the positively charged hydroxide layers. For example, in the case of carbonate anions, the oxygen atoms in the interlayer are located at the sites around the symmetry axes, which pass through the hydroxyl ions of adjacent brucite-like layers. The carbonate group is oriented in parallel to the hydroxide layer with its three oxygen atoms sitting at three adjacent sets of hydroxyl sites. The hydroxyl groups interact with carbonate directly or via intermediate H_2O through hydrogen bridges: $\text{OH}^--\text{CO}_3^{2-}-\text{HO}$ or $\text{OH}^--\text{H}_2\text{O}-\text{CO}_3^{2-}-\text{HO}$.⁶² The interlayer arrangement of other anions, such as hydroxide, nitrate, and chloride is similar to that of carbonate.

Different from those inorganic anions, the orientation of organic compounds is related to their concentrations and reaction temperature. They may form monolayer, bilayer, or partially overlapped layers. Particularly in the case of carboxylate anions, the carboxylate group is normally anchored to the brucite-like layer, while the hydrocarbon chain is tilted at a certain angle.⁸⁶

2.2.4 Water content in LDHs

Water molecules are normally located in those sites of the interlayer region that are not occupied by the anions. Generally, they are held through the formation of hydrogen bonding with the brucite-like layer and/or with interlayer anions. Kagunya et al. show that the interlayer water molecules are not fixed in one position but instead are able to rotate and to move freely about hydroxide oxygen sites.¹¹² However, it is possible to calculate the maximum amount of H_2O on the basis of the number of sites present in the interlayer, assuming a closely packed configuration of oxygen atoms, and subtracting the sites occupied by the anions⁶².

Several formulas can be used to calculate the amount of interlayer water (m value) as follows:

a) According to Miyata,¹¹³ $m = 1 - N x/n$;

where : N is the number of sites occupied by the anion;

n is the charge of the anion;

$$x = M^{III}/(M^{II}+M^{III})$$

b) According to Taylor,¹¹⁴ $m = 1 - 3 x/2 + d$, where $d = 0.125$;

c) According to Mascolo et al.,¹¹⁵ for MgAl-LDHs, $m = 0.81 - x$.

Based on the assumption that water molecules occupy the vacant sites, in all cases, an increase in x (and therefore in the counter anion) causes a decrease in the calculated amount of water. The interaction between water molecules and interlayer anions may also cause an expansion in the thickness of the interlayer region. It has been found that the water content can also be affected by the environment. For example, higher humidity leads to more intercalated water. However, the value of m is difficult to be evaluated accurately as LDHs physically adsorb water molecules on their surface as well.

2.3 Synthesis Methods of LDHs

The synthesis of LDHs is relatively simple and can be carried out via various routes, such as coprecipitation, hydrothermal treatment, anion exchange, reconstruction, and sol-gel methods. In general, the choice of synthesis method affects the properties of the final products, such as crystallinity, particle size, porosity and morphology, etc. A note must be taken that carbonate contamination in the interlayer gallery is quite common when LDHs intercalated with anions other than carbonate are intended to be prepared. In the following subsections, various methods are to be discussed briefly.

Besides the aforementioned commonly adopted methods, there has been recent advancement in the synthesis of LDHs with novel properties and functionalities towards specific applications. These synthesis routes often engage advanced materials fabrication

techniques coupled with the control of the chemical and morphological complexity and diversity. A brief collection of such examples is provided at the end of this section.

2.3.1 Co-precipitation method

This method is most widely used to synthesize various kinds of LDHs owing to its simplicity and ease of scaling up. Due to a higher thermodynamic stability of LDH phases compared to their simple metal hydroxide counterparts, the formation of LDHs are strongly favored under the synthesis conditions with mixed metal cations.¹¹⁶⁻¹¹⁷ In order to precipitate metal cations, it is necessary to carry out the precipitations under the conditions of supersaturation, which is usually reached by physical (e.g., evaporation) or chemical (e.g., variation of pH) methods. In the case of LDH preparation, the pH variation method has been most frequently used. In particular, it is necessary to precipitate at a pH higher than or equal to the one at which the more soluble hydroxide precipitates.¹¹⁸ Practically, a pH range of 8-10 is used for the preparation of LDHs.⁶² There are three methods of coprecipitation reported in the literature.

- 1) Titration of the metal cation solution with a base solution (variable pH method). This method may produce wide distribution of particle size and degree of crystallinity. As pH of solution keeps on increasing with the addition of the base solution, the cation whose hydroxide phase is less soluble will first precipitate at lower pH. This initial precipitate will then convert to the final LDH product, by diffusion of the other cation into the brucite-like layers upon increasing of pH.¹¹⁷
- 2) Coprecipitation at constant pH and low supersaturation. In this method, the pH is controlled by the slow addition of two diluted streams into a single container; the first stream contains the mixed metal cation solution and the second one the base solution. Low supersaturation conditions usually give rise to precipitates that are more crystalline due to sufficient time allowed for crystal growth upon nucleation.¹¹⁹⁻¹²⁰

- 3) Coprecipitation at constant pH and high supersaturation. The metal cation solution is added to the base solution. Hence, the system is suddenly exposed to high supersaturation conditions, so that the rate of nucleation is higher than that of crystal growth, which results in a large number of small sized particles.¹²¹

After coprecipitation, aging step is usually applied to improve the crystallinity of LDHs through dissolution-reprecipitation process.^{62, 122-124} During aging process, the temperature of the mixture is normally kept above the room temperature, i.e. 60-80 °C for a number of hours in order to achieve a better crystallinity. The aging process can also be carried out at an elevated temperature and a pressure higher than the atmospheric pressure, which is the so-called hydrothermal treatment as discussed in the following subsection.

2.3.2 Hydrothermal treatment

This process is in fact a post-treatment for the coprecipitation method in order to obtain uniform and well-dispersed suspension of LDHs with high crystallinity.¹²⁵ The hydrothermal treatment mainly concerns the aging of freshly precipitated mixed hydroxides (in the presence of anions to be intercalated) or the as-prepared amorphous LDHs at temperatures of usually greater than 373 K and pressures of greater than one atmosphere.¹²⁶⁻¹²⁷ In addition, physical mixtures of the metal oxides dispersed in aqueous solutions dissolved with targeted anions can also be treated hydrothermally to obtain LDHs.^{126, 128}

2.3.3 Anion exchange method

This simple method is widely used to prepare LDHs with desirable interlayer anions based on the well-known anion exchange property of LDHs.¹²⁹⁻¹³⁰ The anion exchange process is carried out by dispersing the precursor LDHs in a solution containing an excess of the desired anions that are to be intercalated. Based on the systematic study by Miyata et al., the sequence of exchange for inorganic anions was listed as follows:¹³⁰

The above sequence is associated with the affinity of the anion with the brucite-like layer. Carbonate anion is most difficult to be exchanged out by other anions due to its high affinity to the brucite-like layers. On the other hand, nitrate and chloride anions are relatively easier to be exchanged out from the interlayer. LDHs containing nitrate or chloride anions are, therefore, the most suitable precursors for the preparation of new LDHs with bulky and complex anions such as carboxylic acids, polyoxometalate anions and other macromolecules.

One prominent application that utilizes the anion exchange property of LDHs is the anion-scavenging ability of LDHs to remove hazardous substances from the environment. Some examples are the sorption of herbicides,¹³¹ oxometalates,¹²² removal of chromate ions,⁸² vanadates,¹³³⁻¹³⁵ and the other organic pollutants in wastewater, such as substituted phenols,¹³⁶⁻¹³⁷ chlorinated compounds,¹³⁸ and surfactants.^{111, 139}

2.3.4 Reconstruction method

This method is based on the unique “memory effect” of LDHs which was first reported by Miyata.¹⁴⁰ It was found that the mixed magnesium aluminum oxide obtained by calcination of MgAlCO₃-LDH at 500-800 °C rehydrated and transformed back to a LDH phase in the presence of anions. This method provides an effective route to generate LDH containing desired inorganic and organic anions, and to avoid the incorporation of competing inorganic counter anions.¹⁴¹⁻¹⁴⁴ However, the contamination by carbonate anions from atmospheric CO₂ remains a problem, even if the reconstruction is performed in a nitrogen atmosphere, due to the high affinity of the mixed metal oxides for carbonate anions, especially in the alkaline solution, where CO₂ is converted to carbonate. Some examples using this method are the intercalation of polysaccharides,¹⁴⁵ indomethacin,¹⁴⁶ and ibuprofen anion⁵ into LDHs of various metal compositions.

2.3.5 Sol-gel method

This process involves formation of a mobile colloidal suspension (sol) and then gel due to internal cross-linking. Materials prepared by this technique exhibit good homogeneity, relatively good control of stoichiometry, high specific surface area, and porous characteristics.¹⁴⁷

The LDH formed by this method is the result of the hydrolysis and polymerization of metal alkoxide solutions^{119, 147-149}. The alkoxides are first dissolved in organic solvent and refluxed. To this solution, water is slowly added, causing cross-linkage to occur and subsequently to LDHs. Comparison study of NiAl-LDHs prepared by sol-gel and coprecipitation methods has been carried out by Jitianu et al.¹⁵⁰ and Prinetto et al.¹¹⁹ It was found that synthesis conditions have to be carefully controlled by considering the extraordinary reactivity of the precursors. The Al(acac) was found to be the preferred source of Al³⁺ cations to obtain pure and well-crystallized MgAl-LDHs. The maximum Mg²⁺/Al³⁺ ratio obtained can reach 4.29, which is higher than that generally reported for LDHs prepared by the coprecipitation method. Materials prepared by sol-gel route show different textural and morphologic properties compared to those obtained by classical coprecipitation technique. Prinetto et al. found that samples prepared by coprecipitation method were aggregates of fibrous particles with lengths up to 300 nm and each filament appeared to be constituted by a large number of small particles in a range of 2-4 nm.¹¹⁹ The features of the sol-gel prepared samples showed marked difference, with aggregates of rounded crystallites of 10-15 nm and 2-4 nm for MgAl-LDH and NiAl-LDH, respectively.

2.3.6 Recent advancement in modified synthesis of LDHs with novel properties

2.3.6.1 Synthesis of LDHs with different morphologies

The synthesis methods discussed in the earlier parts usually produce platy hexagonal LDH crystallites. Recently, there has been considerable attempt for modification of synthesis

methods in order to generate LDHs with new morphologies. For instance, Li et al. synthesized curved LDH crystallites with their growth in both a and c directions inhibited by using chitosan as modifiers.¹⁵¹ Fibrous morphology of CoTi-LDH was obtained by a careful control of aging time and the percentage of titanium,¹⁵² as shown in Figure 2-3a. The utilization of anionic surfactants also leads to different LDH morphology. For example, LDHs produced in a water-in-oil emulsion which consists of octane, water and sodium dodecyl sulfate (SDS) exhibit a floccule or fiber-like morphology.¹⁵³ Hu and O'Hare reported the controlled synthesis of LDHs in a similar water-in-oil microemulsion system of isooctane, water and SDS.¹⁵⁴ Nanometer sized LDH platelets can be fabricated from this system. Further modification of the microemulsion using triblock copolymers during crystallization allowed different growth orientations to occur which led to the formation of belt-like LDHs (Figure 2-3b).¹⁵⁴ Another investigation on polyoxyethylene sulfate intercalated MgAl-LDH demonstrated the formation of rigid LDH nanospheres of approximately 200 nm in diameter.¹⁵⁵ A recent report by Prevot et al. demonstrates the formation of flower-like NiAl-LDH by one-pot hydrothermal method in the presence of glycine and under highly alkaline condition (Figure 2-4).¹⁵⁶

Figure 2-3. SEM images of (a) fibrous Co-Ti LDH with 27% Ti and (b) the belt-like LDH produced in the presence of PEO-PPO-PEO triblock copolymer. Images (a) is reprinted from [152]. Copyright 2005, with permission from Elsevier; and (b) is reprinted from [154]. Copyright 2005, with permission from The American Chemical Society.

Figure 2-4. SEM images of flower-like NiAl-LDH at different magnifications. Reprinted from [156]. Copyright 2009, with permission from The American Chemical Society.

Considerable attention has also been paid to the formation of thin films of LDHs using delaminated LDH sheets as the building unit. As the first step, the exfoliation process of LDHs was usually carried out in the presence of organic solvents at elevated temperatures. For example, Jobbagy and Regazzoni reported exfoliation of DS-intercalated LDHs in chloroform and toluene.¹⁵⁷ Venugopal et al. successfully delaminated DS-intercalated LDHs by refluxing in alcohols, in particular 1-butanol.¹⁵⁸ Sasaki et al. reported the exfoliation of LDHs with varied host elements such as CoFe-LDH,¹⁵⁹ MgAl-LDH,¹⁶⁰ and CoAl-LDH¹⁶⁰ in formamide. After the successful delamination and generation of a stable suspension of single-layer LDH sheets, evaporation of the solvent from the suspension on certain substrates results in the restacking of LDH platelets and hence forming LDH nanofilms.¹⁶¹ It has also been demonstrated that by a layer-by-layer (LBL) stacking of positively charged LDH sheets with the anionic polymer (e.g., poly-4-styrene sulfonate, PSS) through electrostatic interactions, formation of smooth LDH films either on flat substrates¹⁶²⁻¹⁶³ or on spherical templates¹⁶⁴ can be attained. For example, Figure 2-5 shows the images of oxide shell obtained after calcination of LDH nanosheets stacked on to PSS beads.¹⁶⁴

Figure 2-5. SEM (a) and TEM (b) images of the oxide shell obtained after calcination of LDH nanosheets stacked on to PSS beads by LBL method. Reprinted from [164]. Copyright 2006, with permission from The Royal Society of Chemistry.

The formation of continuous LDH films can also be achieved by direct self-assembly of well-dispersed colloidal suspension of LDH nanoparticles on flat substrates. The conventional synthesis method for LDHs by coprecipitation at varied or constant pH normally results in polydispersed particles that are unstable and easily aggregate in the solution phase. The suspension containing these aggregates is not possible to be used to prepare a thin film. Several groups of researchers have developed different methods to synthesize transparent colloidal LDH suspensions with narrow particle size distributions. Duan and co-workers employed separate nucleation and aging steps to obtain LDH nanoparticles with a narrow distribution.¹⁶⁵ Xu et al. applied a quick coprecipitation followed with hydrothermal treatment of the as-prepared LDHs after the removal of the electrolytes from the mother liquor.¹²⁵ On the other hand, Gardner et al. carried out a direct synthesis of colloidal alkoxide-intercalated LDH nanoparticles in alcohol as the reaction medium.¹⁶⁶ The casting of the colloidal suspensions of these stable, well dispersed LDH nanoparticles results in the formation of continuous and transparent thin film consisting of highly oriented and densely packed LDH nanoplatelets as shown in Figure 2-6.¹⁶⁷⁻¹⁶⁸

Figure 2-6. SEM images of the surface (a) and the edge (b) of the self-supporting LDH thin film. Inset shows high resolution image of the edge view. Reprinted from [168]. Copyright 2007, with permission from The Royal Society of Chemistry.

2.3.6.2 Polymer encapsulated LDHs

Drug intercalated LDHs have been widely synthesized and studied for controlled drug release. The review on this topic will be provided shortly. For pharmaceutical applications of drug-LDHs in controlled release, coating of drug-LDH particles with biodegradable polymers helps protect the compounds from the harsh environment, as well as slower the drug release rate. Due to its hydroxide framework, LDH is readily soluble under acidic condition. Therefore, for drug-LDH hybrids intended for oral administration, a quick and complete release of drug molecules would occur once the compounds enter the stomach, which has pH value of around 1-3.¹⁶⁹ Further, the anion exchange occurs rapidly in a medium containing electrolytes. Therefore, even in a neutral medium, an initial rapid burst release often occurs.¹⁰⁰ To overcome these issues, Du et al. encapsulated LDH particles into vesicles which were formed by the assemblies of the amphiphilic molecules, consisting of unilamellar or multilamellar closed bilayer (Figure 2-7).¹⁷⁰ It was found that the positively charged LDH particles induced spontaneous formation of vesicles in the mixture of dodecyl carboxyl betaine ($C_{12}BE$) and sodium bis(2-ethylhexyl) sulfosuccinate (AOT).

Figure 2-7. Negative stained TEM images of (a) MgAl-LDH nanoparticles and (b) the composite of LDH encapsulated in vesicle of $C_{12}BE/AOT/water$ system. Reprinted from [170]. Copyright 2007, with permission from The American Chemical Society.

In another report, Mangiacapra et al. succeeded in incorporating MgAl-LDHs into maleic anhydride-modified polycaprolactone.¹⁷¹ Tammaro et al. prepared the same polymeric composite materials containing MgAl-LDH intercalated with chloramphenicol succinate.¹⁷² They showed that the release process from these composites consisted of two stages: initially there is a rapid burst giving rise to a release of a small fraction of drug; and this stage is followed by a much slower release over a longer period of time. Such a release behavior was remarkably different and slower than that from a sample in which the drug molecules were incorporated into the polymeric.¹⁷² Another work on encapsulation of MgAl-LDHs containing diclofenac into Eudragit® S or Eudragit® L polymers were reported by Ambrogio et al.¹⁷³ These two polymers form enteric coatings on LDH particles and would protect them from dissolution in acidic environment.

2.3.6.3 LDHs with luminescence properties

Recently, there has been significant development on materials with multi functionalities for enhanced performance and capability. Among those engineered properties, luminescence has gained a lot of attention and there has been dramatic growth in the use of fluorescence for cellular and molecular imaging.¹⁷⁴⁻¹⁷⁶ Fluorescence imaging can reveal the

localization of intracellular molecules, sometimes even at a level of single-molecule detection, which is very useful in pharmaceutical and biomedical fields.¹⁷⁷⁻¹⁷⁹

As a group of materials with versatile applications, LDHs introduced with luminescence property is worth studying as it may improve their potential applications as drug carriers as well as biosensors. A number of studies have been carried out to incorporate certain phosphors in the interlayer galleries of LDHs to render them with fluorescence features. Li et al. intercalated $[\text{Eu}(\text{EDTA})]^-$ chelate via anion exchange method.¹⁸⁰ Another work on the incorporation of different types of Eu(III) complexes was carried by Sarakha et al.¹⁸¹ In their study, ZnAl-LDHs were intercalated with $\text{Eu}[\text{tris}(\text{dipicolinate})]$, $\text{Eu}[\text{diethylenetriamine pentaacetate}]$, and $\text{Eu}[\text{disulfonated bathophenanthroline tris}(\text{dibenzoylmethanate})]$. It was concluded that the host-guest interactions in the hybrid materials affect the intensity parameter (Ω_2) value as well as the quantum efficiency.¹⁸¹ Intercalation of dipicolinate complexes of Ce(III) and Eu(III) into LDHs with different host metal cations, such as Zn^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} as M^{II} and Al^{3+} , Cr^{3+} as M^{III} was reported by Chang et al.¹⁸²

Some organic chromophores such as green fluorescent protein was also successfully intercalated in MgAl-LDHs and the cellular transfection study was carried out.¹⁸³ The cell lines used were able to internalize the hybrids with up to 90% transfection efficiency. Lang et al. incorporated porphyrin (5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin) in nano-ordered oriented film of LDH containing dodecyl sulfate anion.¹⁸⁴ The photophysical properties of porphyrin, such as fluorescence and the excited-state dynamics were studied extensively.

Besides intercalating phosphors in the interlayer space, there are a few reports on incorporating lanthanide cations in the lattice of brucite-like sheets of LDHs. A novel Y^{3+} containing MgAl-LDH was reported by Fernandez, et al.¹⁸⁵ The amount of Y^{3+} was varied and the crystal structure was studied upon heat treatment. The reconstruction behavior after calcination was also examined. Birjega et al. prepared a series of rare earth elements (Y^{3+} ,

Dy^{3+} , Gd^{3+} , Sm^{3+} , La^{3+}) doped MgAl-LDHs by coprecipitation method.¹⁸⁶ Their corresponding mixed oxides obtained by calcination were studied in detail and their catalytic activities on the cyanoethylation of ethanol with acrylonitrile were investigated. Das et al. prepared MgAlCe-LDH also by coprecipitation method with varying Al : Ce molar ratio.¹⁸⁷ The crystallinity of the products and the textural properties of its oxide were investigated. A novel red light-emitting material, Eu(III)-containing $\text{Ca}_3\text{Al}_2\text{O}_6$ oxide derived from CaAlEu-LDH precursor was recently prepared by Gao et al.¹⁸⁸ It was revealed that photoluminescence intensity was dependent on the calcination temperature and the content of Eu^{3+} in the lattice.

2.4 Development of Layered Double Hydroxides for Pharmaceutical Applications

Up to date, tremendous efforts have been put to synthesize bioactive molecules-intercalated LDHs (bio-LDHs) for pharmaceutical applications. As mentioned earlier, various kinds of drugs and active biochemical compounds such as amino acids, peptides, vitamins, sugars, DNA and ATP, mostly in their anionic form, have been successfully intercalated. The construction of these bio-LDH hybrid systems has so far been achieved by three strategies: (1) electrostatic interaction for those that are negatively charged/in anionic form,^{86, 189} (2) hydrogen bonds for non-ionic compounds that are rich in hydroxyl groups,¹⁰³ and (3) encapsulation with an anionic micelles for poorly water-soluble compounds.⁶ In principle, there are several incentives for the intercalation of these bioactive molecules in LDHs, including i) protection and stabilization of active biomolecules, ii) controlled drug release, and iii) effective cellular delivery. The applications of LDHs in these aspects will be discussed separately in the following subsections.

2.4.1 LDHs as stabilizers and protecting agents

LDHs can act as stable host matrixes for storage and stabilization of labile bioactive substances. Naturally, many vitamins and amino acids are rapidly degraded by light,

temperature, oxygen, alkali metal, etc. In fact, their quick degradation greatly limits their application as active ingredients in cosmetics, foods, and drugs. By storing them in the interlayer space of LDH, they will be protected against detrimental environment. For example, ascorbic acid (vitamin C) is very sensitive to oxidation in the presence of oxygen and is easily degraded upon exposure to light. Choy and Son reported the synthesis of ascorbate-LDH and α -tocopherol acid succinate-LDH (vitamin E-LDH) hybrids.¹⁹⁰ This study clearly showed that through hybridization, vitamins can be stabilized in the interlayer space of LDHs while preserving their chemical and functional properties. Furthermore, the stabilized vitamins can be intentionally discharged from the hybrids through either anion exchange reaction or dissolution of the LDH framework. Another study on the effect of immobilization of chiral non-essential amino acid L-Tyrosine has been conducted.¹⁰⁷ The aim was to study the rate of racemization process of the enantiomer L-Tyrosine with and without intercalation in LDH interlayer. As a chiral compound, L-Tyrosine is very susceptible to racemization process stimulated by heat, sunlight, and ultraviolet light. In addition, it can be easily oxidized to a quinone. The results showed that the immobilization of this enantiomer in LDHs matrixes (NiAl-, MgAl-, and ZnAl-LDH) may reduce the rate of both processes. The specific optical rotation of pristine L-Tyr decreased rapidly by 40-50% while the intercalated L-Tyrosine only showed a slight decrease (less than 5%) in its optical activity under exposure to heat, sunlight, and ultraviolet light. It is most likely that the obstruction of light and heat penetration by the hydroxide layers accounts for this improvement. The intercalation of different types of amino acids like glycine, aspartic acid, glutamic acid, leusin, and phenilalanine into the interlayer regions of LDHs have been reported as well.^{104-105, 191}

2.4.2 LDHs for controlled drug release

Drug formulations with controlled release features possess great advantages and have lately gained much interest.¹⁹²⁻¹⁹⁴ LDHs, due to their layered structure, have been used as

carriers for many clinically important drugs. Besides the aforementioned benefits of high intercalation capacities, stabilization, and protection effects for drugs, the systems of drug intercalated LDHs (drug-LDHs) also introduce another important application in controlled and sustained drug release. As a result, advantages such as prolongation of drug action, minimization of side effects of drugs, reduction of administration frequency through the prolonged half-life time, and better patient compliance could be possibly realized. Many studies have been carried out on the intercalation of various drugs in LDHs and subsequent *in vitro* drug release kinetics. For example, such work has been reported for non-steroidal anti-inflammation drugs (NSAIDs), which may cause adverse gastric and duodenal ulcers formation at high dosages. In particular, ibuprofen,^{5, 195-196} fenbufen,¹⁹⁷ naproxen,^{101, 198} diclophenac,^{100, 196, 199} indomethacin,^{2, 146} ketoprofen,¹⁹⁶ salicylic acid,¹⁰¹ and camptothecin⁶ have been intercalated into various LDHs. After successful intercalation, drug released was examined *in vitro* at different pH to simulate gastrointestinal environment.

It was found that the drug release profile is dependent on both the compositions of the hydroxide layer and the type of drug anions. The release study of ibuprofen at pH 7.5 from MgAl-LDH hybrid results in 60% release within 20 min and 100% release within 100 min.¹⁹⁵ Li, et al. reported the release study of fenbufen from MgAl-LDH and LiAl-LDH at pH 7.8.¹⁹⁷ The release profile of LiAl-fenbufen-LDH shows a high initial drug release rate in the initial 10 min and then reaches a constant concentration after 20 min. MgAl-fenbufen-LDH shows a significantly lower release rate in the initial 15 min, followed by almost linear increase with time, with 59% of the drug released after 120min. The release profile of naproxen from ZnAl-LDH with varied Zn/Al molar ratio was reported by Hou, et al.¹⁹⁸ The results indicate that more Al³⁺ leads to slower release rate as electrostatic interaction between LDH layers and the intercalated drug is stronger. The release test of dichlophenac carried out by Ambrogi et al.¹⁹⁹ in pH 7.5 shows that 38% of drug was released after 15 min and 90% after 9 hours, while Khan et al.¹⁰⁰ obtained a faster release at pH 7 with 90% of drug was released within 40

min. Intercalation of a poorly water-soluble drug, such as indomethacin, camptothecin, and ketoprofen into LDH was proven to improve their solubility at low pH. In addition, the release test of camptothecin-LDH carried out at pH 4.8 and 7.2 demonstrates rapid release rate with 95% and 85% release, respectively, within initial 10 min.⁶

Apart from NSAIDs, some other medicines have been successfully incorporated into LDHs. Wang et al. reported the intercalation of 5-fluorouracil, which is an antimetabolic drug used extensively in cancer therapy.²⁰⁰ Because of the adverse toxicity, selecting a proper controlled release system can enhance its anticancer activity and then reduce its toxic effects. The *in vitro* release profile shows rapid release within the initial 40 min, where 65% and 50% of 5-fluorouracil was deintercalated at pH 4 and 7, respectively, followed by a more sustained release upon prolonged time. After 150 min, 87% and 74% of 5-fluorouracil was released from LDH layers.²⁰⁰ In another account, intercalation of a hypertensive drug was also reported. Captopril-intercalated MgAl-LDHs was successfully synthesized by coprecipitation method.²⁰¹ The *in vitro* release tests of Captopril-LDH showed that both the release rate and release percentages significantly decreased with an increasing pH of the medium. At pH 4.6, the release process involved a quick dissolution followed by an anion-exchange process leading to a slower release rate at a later stage. At pH 7.45, a slower and persistent release process was reported and was interpreted mainly based on the anion-exchange process.²⁰¹

To achieve the effectiveness for oral administration, the physiology of gastrointestinal track must be carefully taken into account. The normal pH in the stomach is about 1-3 with 2 hour retention time. In the duodenum, the pH is 4.5 ± 0.5 with 2-3 hour transit time and it rises up to 7.5 ± 0.4 in the lower part of intestine (jejunum and ileum) with 5 hour transit time. Considering the acidic pH values in the stomach and duodenum, an enteric-coating formulation for drug-LDHs is thus required to prevent an immediate release of drugs, since LDHs dissolve rapidly in acidic media ($\text{pH} < 4$). In this regard, several groups reported the surface coated drug-LDHs by enteric polymers.²⁰² The methacrylate copolymers, Eudragit®L,

Eudragit[®]S, and hydroxypropyl methylcellulose acetate succinates (Aquat AS-MF and AS-HF) have been used to for such a purpose.²⁰³ These polymers are insoluble in acidic condition but can be dissolved at pH 6-7. Therefore, they offer the advantage of sustained drug release in the intestine where the drug absorption occurs. In another report, it has been shown that LDHs coated with oleate is also acid resistant as oleate is insoluble in an acid medium.²⁰⁴ As a result, the intercalated drug molecules are protected from stomach acid and safely delivered to the intestine, where they become available upon dissolution of oleate in the alkaline conditions.

2.4.3 LDHs for cellular delivery

One of the great advantages of using LDHs as a nonviral cellular delivery agent is their good biocompatibility and lower cytotoxicity. MgAl-LDHs in particular have been shown nontoxic to mammalian cells and rats.^{1, 41, 44} MgAl-LDHs nanoparticles are found to degrade in the cytoplasm at pH 4-6, resulting in the liberation of cyto-friendly cations, Mg²⁺ and Al³⁺. Furthermore, the slight acidic condition (pH 4-6) in the cytoplasm leads to a slow dissolution of LDHs and therefore, the intercalated biomolecules in LDHs are subsequently released in a sustained manner.

It was reported by Choy et al. that the application of MgAl-LDH nanoparticles as cellular delivery vectors can improve the uptake efficiencies of biomolecules such as ATP,²⁰⁵ folate derivative drugs,²⁰⁶⁻²⁰⁷ DNA,³ and *c*-antisense oligonucleotide (*As-myc*).³ For example, it was found that the transfection efficiency of ATP into cells was enhanced, due to partially dissolved LDH nanoparticles at cellular pH.²⁰⁵ In another study about folate derivative compounds, such as folinic acid and methotrexate (MTX) that are well known as anticancer drug, it was demonstrated that MTX-LDH suppressed the proliferation of tumor cells. Such finding indicated that the delivery of MTX to the tumor cells was noticeably enhanced by hybridization with LDHs.²⁰⁷ In the hybrid system, MTX can be delivered to the tumor cell

membrane without undergoing any premature transformation as LDH matrix can stabilize and protect MTX molecules. Investigation on the efficacy of As-*myc*-LDH hybrid as a therapeutic agent was also demonstrated using human leukemia cells (HL-60 cells). The result showed that the LDH hybrid was able to inhibit leukemia cell growth by 65% while As-*myc* alone could not even enter the cells and had no significant effects.³

In order to achieve an efficient cellular delivery system, the mechanism of internalization of biomolecules together with their carriers must be well understood. A schematic picture that illustrates hybridization and proposed transfer mechanism of the bio-LDH nanohybrids into a cell is presented in Figure 2-8.²⁰⁸

Figure 2-8. Illustration of the hybridization and proposed transfer mechanism of the bio-LDH nanohybrids into a cell. Reprinted from [208]. Copyright 2006, with permission from Elsevier.

When a conventional administration route is used for bioactive molecules, they will distribute randomly in the body with no preference to recognize targeted cells. In order to be

internalized into a cell, LDH nanohybrid must have a particular interaction with the destined cell, which enables it to penetrate through the cell membrane. Basically, by charge neutralization through hybridization between LDHs and biomolecules, it helps facilitate the penetration of the hybrid into a cell via endocytosis (for < 500 nm particles) since it remarkably reduces the electrostatic repulsion between cell membranes which are negatively charged and anionic biomolecules during endocytosis.⁴⁴ Once the bio-LDH hybrid is introduced into a cell, an endosomal compartment is formed, as shown in Figure 2-9.²⁰⁸ Biomolecules are then further released from the endosome into cytoplasm via disrupting endosomal membrane. The rupture could be due to osmotic swelling, which may be induced by slow degradation of LDH layers in acidic environment inside the endosome (pH 4-5). This low pH condition is generally caused by fusion with lysosome to activate enzymes. The quick rupture may enhance escape of the nanohybrids from the endosomal degradation and thus the biomolecule transfection efficiency.

Figure 2-9. Schematic process of release of encapsulated biomolecules: (1) deformation of cell membrane to take up the nanohybrid and endocytosis of nanohybrid into cell; (2) nanohybrid-endosome breaking down; (3): biomolecules released after endosomal breaks. Reprinted from [208]. Copyright 2006, with permission from Elsevier.

Finally, it is worth noting that there is an emerging trend in intercalation of neutral bioactive molecules into LDHs, as many pharmaceutical products are neutral and have complicated structures. This is a challenging topic since some proper modifications to the

conventional synthesis methods must be adopted. It represents a new area in drug-LDH research and more development is expected in future.

2.5 Advantages of Layered Double Hydroxides as Drug Carriers

In comparison with various other materials systems for immobilization of bioactive molecules, LDHs offer many advantages that make this type of material as a potentially promising candidate for controlled release and delivery. Compared to polymeric systems, LDHs are different by their nature as inorganic compounds. Hence, their preparation and handling is relatively easy. Synthesis of LDHs with controlled particle size distribution is also easy to be performed. Moreover, the positively charged and hydroxyl-rich layers of LDHs help drug intercalation without any further modifications. Meanwhile, drug immobilization in polymers often requires certain reaction steps to activate the functional groups, which is harmful to a certain extent, as organic solvents with some toxicity are required to carry out the reactions. Compared to other materials, such as polycations that are also positively charged, LDHs possess low immunogenicity and toxicity. Immobilization using viral vectors and recombinant proteins are very complicated and expensive although they are highly biocompatible and efficient. In comparison with other inorganic particles (e.g. gold, nickel, magnetite, etc.), LDHs offer ready functionality, low toxicity, and high biodegradability. As a layered material, LDHs give better protection and higher loading due to intercalation of bioactive molecules at the molecular level between the hydroxide layers. Immobilization in other inorganic nanoparticles usually only involves the attachment or adsorption of the bioactive molecules to the surface of the particles, thus leading to limited drug loadings and more exposure to the possible harmful environment. Metabolism of inorganic particles is also another issue. Owing to high chemical stability, most of inorganic materials cannot be dissolved and metabolized easily in the cell. This is harmful since they will be accumulated and circulated in the body. However, in the case for LDHs, they are

easily dissolved in acidic environment inside endosome and liberating non-hazardous Mg^{2+} and Al^{3+} ions, if the dosage is properly controlled.

Nevertheless, there are some limitations of using LDHs as carriers. Despite their ability to incorporate bioactive molecules in the interlayer space, it is difficult to intercalate long chained molecules and neutral compounds, of which are common forms of pharmaceutically active compounds.

The second concern may lie in possibility of aggregation of the LDH nanohybrids. LDH particles can easily form large aggregates through hydrogen bonding, Van der Waals interaction, etc. The transfection efficiency would be greatly reduced when these large aggregates are formed. In addition, compared to viral vectors and recombinant proteins, the transfection efficiency of LDH nanohybrids is relatively low. However, if the surface of LDH nanohybrids are modified with proper bio-functional molecules, e.g., antibody proteins, then this gap can be bridged effectively. In addition, surface functionalization with ligands, such as folic acid may give recognition to targeted tumor cells during delivery process, which in general will keep high drug bioavailability at the desired cells.

CHAPTER 3 SYNTHESIS METHODOLOGY AND MATERIALS CHARACTERIZATION TECHNIQUES

3.1 Materials Synthesis

Coprecipitation method was mainly used to synthesize layered double hydroxides in this PhD work. For some experiments, hydrothermal aging was used as a post-treatment following the precipitation of precursors. Briefly, a solution containing two or more cations with a certain molar ratio was added into an alkaline solution containing the desired anion for coprecipitation to occur. Vigorous stirring was usually applied to ensure homogeneity of the reaction mixture. The reaction mixture was bubbled with nitrogen to minimize contamination by atmospheric carbon dioxide. The as-formed precipitate was then aged under elevated temperature for a certain period in order to obtain homogeneous LDH materials of good crystallinity. The effects of following parameters were usually investigated during the materials syntheses:

- a) Type of anions (drug compounds);
- b) Molar ratio of cations;
- c) type of solvent and the composition;
- d) Reaction and aging temperature;
- e) Aging time.

3.2 Characterization Techniques

3.2.1 Powder X-ray diffraction (XRD)

The crystallographic information of the samples was analyzed by powder X-ray diffraction (XRD). XRD technique is primarily used for identification of crystalline materials. In the powder method, the sample is prepared in the form of fine homogeneous powder. As a result, the sample contains a large collection of vary small crystals which could be orientated

in every possible direction relative to the beam of radiation. Therefore, the diffraction rays can be corresponding to all sets of crystal planes. During the specimen preparation, the solid sample is finely ground and shaped into a thin layer with a smooth surface in a noncrystalline sample holder, which is then inserted in the path of X-ray beam. Bruker AXS D8 X-ray diffractometer with Cu K α ($\lambda=1.5406$ Å) radiation at 40 kV and 20 mA was used to collect the diffraction patterns. The 2θ scanning range was from 2° to 70° at a scanning speed of $1.2^\circ \text{ min}^{-1}$.

The relationship between the wavelength of X-ray beam, diffraction angle, and the distance between each set of atomic planes of the crystal lattice is provided by Bragg's equation: $\lambda = 2d \sin \theta$, which λ is the wavelength of X-ray radiation, d is the crystal plane distance, and θ is the diffraction angle.²⁰⁹

The average crystallite size, D_p , is estimated using Debye-Scherrer formula from full width at half maximum (FWHM) of some well-defined and intense peaks as follows:²¹⁰

$$D_p = \frac{0.9\lambda}{FWHM \cos \theta}$$

3.2.2 Fourier transform infrared (FTIR) spectroscopy

The qualitative infrared spectroscopy is one of the most powerful tools to analyze the presence of particular compounds or moieties in the sample. This is achieved by recognizing characteristic shapes and patterns within the spectrum. The generated spectrum is formed as a consequence of the absorption of electromagnetic radiation at frequencies that correlate to the rotational and vibrational motions of specific chemical bonds within a molecule.²¹¹ The KBr pellet technique is widely used for solid sample analysis. In this work, a few milligrams of finely ground sample was mixed with KBr powder with a mass ratio of about 1:100. The mixture was then palletized before the measurement. The measurement was conducted in a Digilab FTS 3100 instrument by collecting 64 scans with a resolution of 4 cm^{-1} at the mid-infrared region ($400\text{-}4000 \text{ cm}^{-1}$). In this work, FTIR was used to analyze the chemical

bonding information of metal-oxygen, metal-hydroxyl, and the characteristic absorption of the surface polymer coating and the interlayer anions including drug species.

3.2.3 Thermogravimetric and differential scanning analysis (TGA, DTA)

Thermogravimetric analysis (TGA) is a technique in which the mass of the sample is monitored as a function of temperature while the sample is subjected to a controlled heating under the controlled atmosphere.²⁰⁹ This analysis is used to study the physical or chemical changes that occur in the sample upon heating. On the other hand, DTA measures temperature difference between the sample and reference material as a function of temperature when both are subjected to the same controlled heating. The DTA curves provide information about the absorbed (endothermic) or evolved (exothermic) energy during a physical process or chemical reaction upon heating of the sample. In this work, TGA and DTA studies were carried out in TA Instrument SDT Q600 with a heating rate of 10 °C min⁻¹ and air flow rate of 200 ml/min from room temperature up to 800 °C.

3.2.4 Elemental analysis

Elemental analysis provides the weight percentages of carbon, nitrogen, hydrogen and sulfur in the solid sample. During the measurement, the bulk solid sample is combusted to convert all these elements to their corresponding gaseous species, CO₂, H₂O, N₂, and SO₂. The mixture of the gas is further separated by a chromatographic column and the outlet gaseous species are analyzed by a thermal conductivity detector (TCD). The weight percentages of carbon and nitrogen in this work were determined in an Elementar vario elemental analyzer.

3.2.5 Inductive coupled plasma-atomic emission spectroscopy (ICP-AES)

The principle of this analytical technique is based on the excitation of electrons of the element by electronically generated plasma to the higher level of energies above the ground state. Plasma is a neutral gas containing significant number of both positive and negative ions or free electrons.²¹² When electrons are falling back to its normal state, a photon radiation with a particular wavelength corresponding to the difference in the energy levels is emitted. There is an empirical correlation between the power of the emitted radiation of some particular wavelength and the quantity of the corresponding element in the sample. Such information is then used to generate mass spectrometry for quantitative analysis of inorganic components in a solution. In this work, the solid samples were first dissolved in nitric acid solutions to obtain clear solutions containing metal cations of LDHs. The measurement of cation concentrations was performed in a Perkin Elmer ICP Optima 2000DV using Argon plasma as excitation source.

3.2.6 UV-vis spectroscopy

UV measurement technique is widely used to determine concentration of a compound in the solution, as well as to provide information about molecular structure and functional groups.²¹³ The principle of this analysis is based on the fact that electrons could be excited by absorbing electromagnetic radiation at wavelength in the UV-visible region (100-400 nm). According to the Beer's law, there is a relationship between the observed absorbance, concentration of the species to be measured, their optical properties, and the path length of the sample.²¹² Such a relationship can be expressed by a linear equation: $A = \varepsilon C$, where A is absorbance, ε is absorption coefficient that consists of absorptivity (a) and the length of path (b), and C is the concentration. The value of absorption coefficient ε can be determined from the slope of the calibration curve using a set of solutions of standard concentrations. Shimadzu UV2450 UV-Vis spectrophotometer with a halogen lamp as light source was used

to determine the amount of intercalated drugs in our samples after being dissolved or exchanged from LDHs in this work.

3.2.7 Fluorescence spectroscopy

Luminescence arises from the emission of photons from a substance that is at electronically excited states. According to Jablonski's diagram, there are many ways for photon radiation to take place.²¹⁴ When the excited electrons return from the lowest vibrational level of the first singlet state to the ground state, it is known as fluorescence. A few prominent characteristics of fluorescence emission are Stokes' shift and mirror image rule. The energy of the fluorescence emission is typically less than that of the absorption. Hence, it usually requires excitation wavelength in the UV range while the emission occurs in the range of visible light, based on Stokes's rule. The mirror image rule results in a symmetric nature of the excitation and the emission spectra since the spacing of the vibrational energy levels of the excited states is similar to that of the ground state. Shimadzu RF-5301 PC spectrofluorophotometer with a 150W Xenon arc lamp was used to observe photoluminescence spectra of our Tb³⁺ containing LDHs. During the analysis, samples in a fine powder form were first dispersed in a solvent (e.g., methanol). Ultrasonification was applied to the mixture to obtain a homogeneous suspension. The emission spectra were collected at different wavelengths, corresponding to the maximum wavelength obtained from the excitation spectra.

Fluorescence lifetime (τ) and quantum yield (Q) are the most important parameters to indicate the performance of fluorophores. The lifetime of the excited state is the average time that a molecule spends in the excited state prior to returning to the ground state. It can be obtained by the reciprocal of the linear curve plotting the logarithm of the fluorescence intensity against the time.²¹⁴ Quantum yield is the ratio of the number of photons emitted to the number absorbed. It is calculated as: $Q = Q_s \frac{I}{I_s} \frac{OD_s}{OD} \frac{n^2}{n_s^2}$, where Q_s is the quantum yield

of a standard reagent, I is the integrated intensity, OD is the optical density, and n is the refractive index.²¹⁴ Both parameters are strongly dependent on the environment where the fluorophores reside in, as it may promote deactivation of the excited fluorophores. This phenomenon results in the decrease of fluorescence intensity, which is called quenching. The time-domain lifetime of our samples was measured using Fluorolog Jobin-Yvon SPEX to obtain the plot of fluorescence intensity decay against time.

3.2.8 Transmission electron microscopy (TEM)

TEM is a technique to generate two-dimensional images from a specimen by illuminating it with a highly focused and monoenergetic electron beam in vacuum. The transmitted electrons form images on a fluorescent screen or captured by a digital camera, which provide morphological features at low resolution and lattice/atomic arrangements at high resolutions for the nanostructured materials.²¹⁵ In addition, this technique can be very powerful in providing elemental/chemical information of the specimen if coupled with energy dispersive X-ray spectroscopy (EDX) or electron energy loss spectroscopy (EELS). Furthermore, in combination with the scanning transmission electron microscope (STEM) technique, the EDX and EELS data can be collected with a nanometer resolution, which makes possible the construction of detailed elemental maps of the nano-object under study.²¹⁶

During the specimen preparation, a few milligram of the powder samples was dispersed in a volatile solvent, e.g., acetone or ethanol. The mixture was then ultrasonicated to disperse the particles well to form a homogeneous suspension. A few drops of suspension were spread on the copper grids coated with a carbon film. The observation was carried out in a JEOL 3010 electron microscope with a LaB₆ filament, operated at an accelerating voltage of 200 kV.

3.2.9 Scanning electron microscopy (SEM)

In SEM analysis, a narrow electron beam produced by the filament at an accelerating voltage of 5-30 kV is focused and moved over the surface of the specimen. Due to a lower energy of electrons, they can only hit a few surface layers of the object, and then being scattered back or causing secondary electrons. Secondary electron images mainly provide information on the morphology, such as shape, size, and surface texture and topography, while backscattered electrons will offer compositional images, with different elements in the sample appearing with different contrast. SEM is frequently applied to have a “quick look” at the general morphology of the solid sample. Despite its convenience in sample preparation, this technique only provides a rough surface feature of the particles at relatively low magnification.²¹⁷ In this work, the SEM images were collected in a JEOL JSM 6700F field emission scanning electron microscope (FESEM) with an accelerating voltage and a beam current at 5 kV and 10 μ A, respectively. Samples in a fine powder form were spread onto the conducting carbon tape or silicon wafer. Due to a non-conductive nature of the LDH samples, sputtering with Pt for around 90 sec was carried out before SEM observation.

3.2.10 X-ray photoelectron spectroscopy (XPS)

XPS is based on the ejection of photoelectron from inner orbital of a target atom as a result of the bombardment of the substance surface by monochromatic X-rays. Upon irradiation, the number of electrons that escape from the materials surface is quantified with their kinetic energies measured simultaneously. Binding energy (BE), which is defined as the attraction energy between the electron and the nucleus, is obtained from the difference between X-rays photon energy and kinetic energy of the escaped electron. BE is specific for a given electron in a given element. In addition, BE of a given electron in the same element changes with a specific chemical environment. Therefore, this method can be used for surface element and chemical species identification. As the number of electron ejected is

proportional to the quantity of the element, the peak areas can be used (with appropriate sensitivity factors) to determine the compositions of the surface.²¹⁸ The XPS spectra presented in this report were collected from Kratos-Axis Ultra System with monochromatic Al K α X-ray source (1486.7eV) operating at 15 kV and a pressure of 10⁻⁹ torr. Samples were finely ground before being mounted onto the adhesive tape. BEs of all elements were corrected by referring to the Au 4f peak (BE of 83.6 eV) that was sputtered on the samples under study.

3.2.11 Particle size analysis

A colloidal dispersion is known to be able to scatter a beam of light. This light scattering behavior, in turn, offers an opportunity for determining the size of colloidal particles as the intensity of the scattered light has a proportional correlation with particle size.²¹⁹ The method of dynamic (quasi-elastic) light scattering (QELS) has been widely used, especially to determine particle sizes below 1 μm . It utilizes a coherent, monochromatic, and intense laser as the light source for detecting a wide range of particle shapes and size at high accuracy. In our study, a dilute homogenous suspension was prepared before being analyzed in a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation).

3.2.12 Zeta potential analysis

A great majority of colloidal materials is charged due to surface dissociation, ion adsorption on the particle surface, and crystal lattice defects. Therefore, a homogenous colloidal suspension is stabilized by Brownian motion of particles due to repulsive interactions among the charged particles. The magnitude of the surface charge is represented by Zeta potential (ζ).²¹⁹ The Zeta potential of the samples homogeneously dispersed in a solvent was measured in a PALS Zeta Potential Analyzer (Brookhaven Instruments Corporation) in this work.

3.2.13 Surface area and porosity measurement

The physical adsorption/desorption of liquid nitrogen as adsorbate at 77 K onto solid surface sites based on BET (Brunauer-Emmet-Teller) method can be used to measure the specific surface area of solid samples. The BET method extends Langmuir's monolayer theory to multilayer adsorption since in the real experiment it is impossible to obtain monolayer coverage. The effectiveness of BET theory is that it enables an experimental determination of the number of adsorbate molecules required to form monolayer, hence, making it a popular approach to calculate specific surface area.²²⁰ The average pore size analysis is determined by BJH (Barrett-Joyner-Halenda) method using the desorption curve of the sorption isotherm. The sorption analysis of the samples was carried out using Quantachrome Autosorb 6B instrument after certain period of degassing.

CHAPTER 4 DIRECT CONTROL OF DRUG RELEASE BEHAVIOR FROM LAYERED DOUBLE HYDROXIDES THROUGH PARTICLE INTERACTIONS

(This chapter is reproduced with permission from “Gunawan, P. and Xu, R., *Direct control of drug release behavior from layered double hydroxides through particle interactions in solid aggregates*. Journal of Pharmaceutical Sciences 2008, 97, 4367-4378.” Copyright 2008, Wiley InterScience)²²¹

4.1 Introduction

In the author's paper in *Journal of Pharmaceutical Sciences*,²²¹ it is said “Recently there has been considerable interest from the pharmaceutical industries in the controlled release drug delivery systems.²²² In particular, it was expected that the market for oral controlled drug delivery may grow annually at more than 9% between 2003 and 2007.²²³ In order to be able to conveniently store and easily transport the pharmaceutical ingredients, the preferred dosage form is often in the solid state such as tablets, capsules, etc. These dosage forms are usually more stable than their liquid or suspension counterparts.²²⁴ As a result, solid matrix materials are frequently applied in controlled delivery systems for preloading and subsequent liberation of drugs. On the other hand, in order for treatment of different types of diseases, the drug input from the solid matrix into the body shall be regulated to mimic various kinetic input models, ranging from a simple instantaneous to complex ones.²²² Moreover, it is expected for more challenges in the design of more effective systems, as the pathological and physiological mechanisms of diseases are further elucidated. With all these regards, it remains as an important research subject for further development in engineering and material sciences to achieve successful controlled delivery systems.

As mentioned earlier in Chapter 2, LDHs represent one of the most important emerging drug delivery systems based on their unique host-guest type structure. The Mg^{2+} and Al^{3+} cations form the most common compositions in LDH host layers since they have low toxicity and good biocompatibility. The introduction of many advantages of drug containing LDHs, such as simple preparation techniques, high drug loadings, enhanced drug stability, sustained drug release, and even improved efficacy of drugs in some cases,^{6, 206, 225} makes this type of inorganic-organic hybrid systems potentially good candidates for controlled release drug delivery systems.

Despite the above commonly recognized facts, the research on the application of drug-LDHs as a delivery system is still at its initial stage. Further investigation of the controlling factors on drug release kinetics is of prime importance towards the development of tunable drug delivery systems, besides the continuing effort to intercalate new drugs into LDHs. Some studies have indicated that the type of host, mainly among LiAl_2 , Mg_2Al , and Ca_2Al cation combinations, poses the major effect on the release profile for the same drug.^{100, 226} However, it seems that such effect varies widely for different drugs. Up to date, there are no straightforward correlations established between the release kinetics and the size of the drug molecules or the charge density of the layers.²²⁶ In the literature, the diffusion (coupled with anion-exchange) and dissolution mechanisms are commonly accepted for the release of drugs from the LDH solid matrix at neutral and acidic conditions, respectively.^{195, 227} Therefore, it is expected that factors which affect the rates of these processes can be manipulated from materials aspects to achieve a desired drug release profile.

From our point of view, besides varying the chemical properties of drug-LDHs, controlling of their morphological features is equally important. In this chapter, our study was focused on the correlation between the crystallite morphology and the state of aggregation of drug-LDHs and their effects on the release of a model NSAID, ibuprofen, from Mg_2Al -LDHs.

4.2 Experimental Methods

4.2.1 Synthesis and characterization of $\text{Mg}_2\text{Al-Ibp-LDHs}$

The coprecipitation method was used to prepare ibuprofen (Ibp) intercalated $\text{Mg}_2\text{Al-LDHs}$. Two types of solvent systems were applied and the samples were aged either at atmospheric or hydrothermal conditions. During the synthesis, 100 mL of solution containing NaOH (0.18 M, Merck, >99.0%) and ibuprofen sodium salt ($\text{C}_{13}\text{H}_{17}\text{O}_2\text{Na}$, 0.06 M, Sigma, >99.0%) in the mixed solvent of ethylene glycol (Merck, >99.5%) and water (volume ratio = 1:1) was first prepared. To this mixture, 20 mL of the mixed solution of $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.30 M, Aldrich, >99.0%) and $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (0.15 M, Fluka, >98.0%) in the same solvent was added drop wise at a constant rate of 0.33 mL/min. The resulting suspension was transferred to a Teflon-lined autoclave and aged under hydrothermal condition at 150 °C for 18 h. To minimize the contamination of CO_2 from atmosphere, the precursor solutions were bubbled with nitrogen at 60 mL/min for about 30 min before the reaction. At the end of aging, the product was washed thoroughly with deionized water and ethanol, filtered, and then dried in the oven at 70 °C overnight. The sample thus obtained is denoted as EG/W-H, where EG/W refers to the solvent system of ethylene glycol and water mixture, and H refers to the hydrothermal treatment. The above synthesis was also carried out in water alone to get the sample W-H, while keeping other experimental conditions the same. For the preparation of samples EG/W-C (in the same mixture of ethylene glycol and water) and W-C (in water alone), the aging was carried out at the atmospheric conditions at 70 °C for 3 days after complete addition of the metal solution. During the aging, N_2 gas was continuously purged to the mixture. In addition, a condensing unit was mounted on top of the precipitation flask to condense the solvent vapor.

The samples were analyzed with XRD, FTIR, ICP, CHN, TGA, TEM, SEM methods that have been described in Chapter 3. The weight percentage of Ibp in solid samples was

measured using UV-vis spectroscopy after a complete dissolution of the solid samples in the mixture of 1.0 M HCl aqueous solution and ethanol of 1:1 volume ratio.

4.2.2 *In vitro* drug release test

The as-prepared Ibp-LDH products were first ground into fine powder using a mortar and pestle. The release of Ibp from these fine powder samples was carried out at a constant temperature of 37 ± 0.5 °C by dispersing 100 mg of dry powder samples in 200 mL of phosphate buffer solution at pH 7.0 (Fluka, NaOH: 0.029 M; KH_2PO_4 : 0.050 M) to simulate the body temperature and the intestinal fluid condition. A constant stirring at 200 rpm was applied. Aliquots of 1 mL were withdrawn from the suspension from time to time, filtered, and diluted to three times. The measurement of Ibp concentration in the filtrate was carried out using UV-vis spectroscopy (Shimadzu 2450) at 264 nm. At the end of release test, the solid samples were collected by centrifuge and dried at 70 °C overnight for further characterization with FTIR and SEM.

4.3 Results and Discussion

4.3.1 Structural and chemical analysis

The successful intercalation of Ibp into the interlayer space of LDHs prepared under all different experimental conditions is evidenced from the XRD and FTIR results shown in Figures 4-1 and 4-2 respectively. The coprecipitation method coupled with aging at either atmospheric or hydrothermal condition is effective for the incorporation of Ibp in $\text{Mg}_2\text{Al-LDHs}$. The XRD patterns of as-prepared samples exhibit a single crystalline LDH phase which can be indexed by the hexagonal lattice with a rhombohedral symmetry.⁶² The diffraction peaks due to (003), (006) and (009) basal planes are observed for all samples and higher basal peaks of (0012), (0015) and (0018) can also be observed for samples EG/W-H and W-H which were hydrothermally treated at 150 °C for 18 h. The d_{003} basal spacing is

obtained in a narrow range of 21.9~22.5 Å based on (003) peak using Bragg's equation. These values are similar to that (21.7 Å) reported by Ambrogi et al. for a Mg₂Al-Ibp-LDHs,¹⁹⁵ but smaller than that (28.5 Å) reported by Gordijo et al. for Mg₃Al-Ibp-LDHs.⁵ Such difference could be attributed to a stronger electrostatic interaction between the brucite-like layers and anions, since more trivalent cations and charge-balancing anions are present in our samples (Mg:Al = 2.0). After deducting the thickness of brucite-like layer (approximately 4.8 Å),²²⁸ the gallery height of the as-prepared samples is 17.1~17.7 Å. Thus, a bilayer arrangement of tilted Ibp anions with their carboxylate groups anchored to the brucite-like layers is suitably proposed, taking into account of the length of Ibp anion ~10 Å.^{195, 229} Among the four samples, EG/W-H has the weakest (110) peak, indicated by the comparison of its intensity between EG/W-H and W-H in the inset of Figure 4-1. This is due to relatively well *c*-oriented aggregation of LDH platelets in the dry powder of this sample,¹⁶⁷ which will be further discussed.

The effects of the solvent systems and aging conditions on the crystallinity of the as-prepared samples are reflected by the intensity and full-width-at-half-maximum (FWHM) of the diffraction peaks in Figure 4-1. It appears that the two solvent systems, water alone or the mixture of ethylene glycol and water (1:1 volume ratio), do not have obvious effect on the crystallinity. An earlier report by Malherbe et al. has indicated that organic solvents with different physical properties, like the type of functional group and polarity, affect the properties of MgAl-CO₃-LDHs.²³⁰ The effect of different solvent systems on the morphology of the samples is to be discussed shortly. On the other hand, it was found that the aging conditions greatly affect the crystallinity of the resultant LDHs. As shown in Figure 4-1, samples EG/W-H and W-H that were aged hydrothermally at 150 °C for 18 h exhibit a much higher degree of crystallinity, compared with samples EG/W-C and W-C that were aged at atmospheric condition (70 °C, 3 days). The estimated crystallite size in the *c*-direction using Debye-Scherrer formula²³¹ is listed in Table 4-1. The thickness of the LDH platelets in

samples EG/W-H (22.1 nm) and W-H (29.8 nm) is approximately twice of those in samples EG/W-C (13.2 nm) and W-C (13.0 nm).

The compositions of the synthesized samples were obtained from the combined ICP, CHN and TGA techniques. The results from these analyses are summarized in Table 4-1. The measured atomic ratio of magnesium to aluminum from 1.94 to 2.07 in these samples follows closely to the starting molar ratio (2.0) in the metal precursor solution, indicating a complete precipitation of metal cations from the solution phase. The absence of both intercalated and surface adsorbed nitrate can be confirmed as nitrogen element was not detected despite a much lower amount of Ibp present in the initial solution. This is also evidenced by the absence of nitrate absorption band in the FTIR spectra of the samples. There are several factors that affect the intercalation of guest anions into LDH: (1) charge density of the anion, (2) host-guest interaction, such as hydrogen bonding or Van der Waals interaction, and (3) guest-guest interaction, such as hydrophobic-hydrophobic interaction.²³² Miyata also first reported that nitrate anion has the least affinity to LDH layers among common inorganic anions, thus it can be easily exchanged out by other anions.¹³⁰ During co-precipitation, the guest anion is precipitated as simultaneously as the formation of hydroxide layers and instantaneously embedded into the as-formed LDH nuclei. In our case, the salt solution was added slowly into Ibp solution under alkaline solution. In this environment, Ibp molecules are completely in the anionic form and are readily intercalated into LDH. Due to slow addition of salt solution, the concentration of nitrate in the suspension is much less than that of Ibp, hence Ibp anions dominate in the interlayer in the early stage of co-precipitation. Hydrophobic-hydrophobic interaction between Ibp molecules may attract free Ibp from the solution to LDH particles. In this way, Ibp can be completely intercalated.

The overall chemical formulas shown in Table 4-1 indicate that Ibp is the main interlayer anions in all the samples prepared in this work. Small percentages of carbonate could be co-present in the interlayer space based on the compositional analysis. The accuracy

of the determined formulas has been confirmed by comparing the measured oxide residue percentages obtained by TGA against the calculated values using the formulas. It has been found that the discrepancy is less than 4%.

Figure 4-1. Powder XRD patterns of as-prepared $\text{Mg}_2\text{Al-Ibp-LDH}$ samples using two different types of solvent systems at atmospheric or hydrothermal conditions, (a) EG/W-H; (b) W-H; (c) EG/W-C; and (d) W-C. The inset displays the (110) diffraction peak of samples (a) EG/W-H; and (b) W-H.

Table 4-1. Samples synthesized with different experimental parameters and their properties, chemical compositions and drug release results.

Sample	T (°C)	Aging time (h)	D_c^a (nm)	D_a^b (nm)	%Mg ^c (wt%)	%Al ^c (wt%)	%C ^d (wt%)	%H ^d (wt%)	%H ₂ O ^e (wt%)	Chemical formula	% Ibp ^f (wt%)	$t_{50\%}^g$ (min)	$t_{90\%}^h$ (min)
EG/W-H	150	18	22	450	11.90	6.39	33.7	6.8	13.6	Mg _{2.07} Al(OH) _{6.14} (Ibp) _{0.93} (CO ₃) _{0.04} ·3.17H ₂ O	44.25	68	160
W-H	150	18	30	300	11.91	6.80	34.6	6.9	14.2	Mg _{1.94} Al(OH) _{5.88} (Ibp) _{0.94} (CO ₃) _{0.03} ·3.15H ₂ O	45.41	18	57
EG/W-C	70	72	13	200	11.97	6.78	34.9	6.9	12.0	Mg _{1.96} Al(OH) _{5.92} (Ibp) _{0.93} (CO ₃) _{0.04} ·2.66H ₂ O	45.91	7	39
W-C	70	72	13	100	12.64	7.12	33.9	6.8	14.6	Mg _{1.97} Al(OH) _{5.94} (Ibp) _{0.92} (CO ₃) _{0.04} ·3.11H ₂ O	44.70	5	58

^aDomain size in c direction, calculated using the Scherrer equation based on (003) diffraction peak.^bThe average diameter of the platelets based on TEM analysis.^cThe weight percentage of magnesium and aluminum obtained from ICP measurement.^dThe weight percentage of C and H obtained from CHN elemental analysis.^eThe weight loss percentage of dry sample during TGA analysis from 25 to 200 °C.^fThe weight percentage of Ibuprofen calculated based on the determined chemical formulae.^gThe time required for 50% of the intercalated Ibp to be released.^hThe time required for 90% of the intercalated Ibp to be released.

Figure 4-2. FTIR spectra of as-prepared Mg_2Al -Ibp-LDH samples using two different types of solvent systems at atmospheric or hydrothermal conditions, (a) EG/W-H; (b) W-H; (c) EG/W-C; (d) W-C; and (e) ibuprofen sodium salt.

The formation of Ibp intercalated Mg_2Al -LDHs is further evidenced from FTIR spectra shown in Figure 4-2. The spectrum of ibuprofen sodium salt (Ibp) is also included for comparison purpose. It is observed that similar spectra were obtained for all the samples. The bands at 1550 and 1399 cm^{-1} are unambiguously assigned to antisymmetric (ν_{as}) and symmetric (ν_{s}) stretching vibrations of the $-\text{COO}^-$ group of Ibp anion respectively, as also observed on Ibp spectrum.^{5, 195} An adjacent peak observed with a slightly lower wavenumber at 1365 cm^{-1} could be attributed to C-H deformation vibration as well as the stretching vibration of the carboxylate group as reported elsewhere¹⁹⁵ while the fingerprints in the region 500-1200 cm^{-1} could also be attributed to C-C skeletal vibrations of ibuprofen molecules.²³³ As discussed earlier, the intercalation of the counter nitrate anion can be ruled out based on

the absence of the absorption peak at 1384 cm^{-1} .²³⁴ It is noted that the $\text{Mg}_3\text{Al-Ibp-LDHs}$ synthesized by Gordijo et al. contain quite appreciable amounts of interlayer NO_3^- anion, especially the one prepared with the co-precipitation method.⁵ The peak at 2950 cm^{-1} indicates the typical $\nu_{(\text{CH})}$ stretching vibrations of Ibp.^{5, 195} The broad band around 3450 cm^{-1} is related to the stretching mode of hydroxyl groups in the brucite-like layers and to the interlayer and physically adsorbed water molecules. The peaks and bands in the lower wavenumber region (i.e. $400\text{-}800\text{ cm}^{-1}$) are due to M-O vibration and M-OH bending in the brucite-like layers.²³⁴ For samples prepared in the presence of ethylene glycol (samples EG/W-H and EG/W-C), it should be noted that ethylene glycol is not intercalated or grafted, based on the observation that the peaks due to the stretching mode of C-C-O in ethylene glycol at around $1081\text{-}1091$ and $1042\text{-}1043\text{ cm}^{-1}$ are absent.²³⁵⁻²³⁷ Although the intercalation or grafting of ethylene glycol in layered hydroxide materials, like LDHs and brucite compounds, have been reported previously,²³⁵⁻²³⁷ those experiments all involved the mixing of the solid materials with pure ethylene glycol in vapor or liquid form. In our syntheses, ethylene glycol was mixed with water and therefore, it is confirmed that water molecules are preferentially intercalated. However, should there be any trace amount of the adsorbed ethylene glycol that is hardly detected by FTIR, it should not introduce any toxicity due to its infinitesimal amount. Moreover, ethylene glycol has a relatively low toxicity as the lethal dosage of pure ethylene glycol is 1.4 ml/kg .²³⁸

The molecular structure of ibuprofen in all samples was examined by UV-vis spectroscopy. Samples containing ibuprofen were dissolved in the mixture of 1M HCl and ethanol with volumetric ratio of $1:1$. The pristine ibuprofen was also prepared in the same way as comparison. As presented in Figure 4-3, the spectra obtained show that there are no changes in the absorption pattern for all samples as well as the ibuprofen salt solution as control. The two main absorption peaks at 264 nm and 272 nm are attributed to the absorbance of the aromatic ring and the carboxylate group of ibuprofen, respectively.²¹² It

should also be noted that there is no shift in the peak position, indicating that the intercalated ibuprofen maintains the original structure, even after hydrothermal treatment.

Figure 4-3. UV-Vis absorption spectra of the as-prepared Mg_2Al -Ibp-LDH samples (EG/W-H, W-H, EG/W-C, W-C) and ibuprofen salt.

4.3.2 Morphological properties of particles and dry powders

The morphological features of the Mg_2Al -Ibp-LDHs observed using TEM and SEM methods are displayed in Figure 4-4 and Figure 4-5 respectively. Overall, it was found that the platelets of sample EG/W-H when deposited on TEM grid were better dispersed and individual platelets can be easily identified, compared with the rest of the samples whose TEM specimen were prepared by the same procedure. Samples aged hydrothermally exhibit different characteristics from those aged under the atmospheric condition. Besides the larger domain size in *c*-direction as obtained from XRD results, the average diameters of the platelets are larger for samples EG/W-H (450 nm, Figure 4-4a) and W-H (300 nm, Figure 4-4b). Furthermore, the platelets display a relatively round shape (Figures 4-4a, b), while

irregularly-shaped platelets are generally observed for samples EG/W-C and W-C with average particle sizes of 200 and 100 nm respectively (Figures 4-4c, d).

Figure 4-4. TEM images of as-prepared $\text{Mg}_2\text{Al-lbp-LDH}$ samples using two different types of solvent systems at atmospheric or hydrothermal conditions, (a) EG/W-H; (b) W-H; (c) EG/W-C; and (d) W-C.

The aggregation states of the two hydrothermally aged samples are quite different from each other. The SEM images (Figure 4-5) displays that sample EG/W-H exhibits dense and larger aggregates with the majority of the platelets stacked in the *c*-orientation (Figure 4-5a). Smooth surface is observed in many local regions on the aggregates, indicating that face-to-face and edge-to-edge aggregation took place during the drying stage to form the oriented powder. In contrast, sample W-H exhibits more random aggregates with interparticle space

(Figure 4-5b). Such difference is consistent with the XRD results in the inset of Figure 4-1 where the in-plane reflection peak (110) for sample EG/W-H is much weaker than that for sample W-H, since the intensity ratio of the basal peak (003) to the in-plane reflection peak (110) is an indication of the sample (particle) orientation.¹⁶⁷ Samples aged under the atmospheric condition also form randomly sized aggregates with obvious inter-particle and inter-aggregate porosity (Figures 4-5c, d). The similar morphologies of drug-LDH powder samples as observed in Figures 4-5b, c, and d are frequently reported by many other authors.^{2,}

195, 227, 239

Figure 4-5. SEM images of the dry powders (after grinding of dry pellets) of as-prepared $\text{Mg}_2\text{Al-lbp-LDH}$ samples using two different types of solvent systems at atmospheric or hydrothermal conditions, (a) EG/W-H; (b) W-H; (c) EG/W-C; and (d) W-C.

Figure 4-6. SEM images of the dry pellets (before grinding) of as-prepared $\text{Mg}_2\text{Al-Ibp-LDH}$ samples using two different types of solvent systems at hydrothermal conditions, (a, b) EG/W-H; and (c,d) W-H.

The effect of EG on the aggregation state of samples EG/W-H and W-H can be further confirmed based on the low-magnification SEM images taken for the dry pellets before grinding. As displayed in Figure 4-6, the surface of sample EG/W-H (Figures 4-6a and b) is smoother, indicating a more orientated arrangement and denser packing of the constituent LDH nanoplatelets. The aggregate state in the pellet of sample W-H (Figures 4-6c and d) is less compact as evidenced from the rough surface with porosity. During the course of grinding, the original packing and aggregation state should be affected to a certain extent. But it is believed that the resulted fine powder of EG/W-H still posses denser packing which gives rise to a slower drug release rate. The more oriented aggregation in sample EG/W-H can be attributed to the presence of EG during the hydrothermal synthesis. As a high boiling alcohol, EG itself may act as a dispersant and stabilizer, thereby limiting the particle agglomeration in

the solution.²⁴⁰ EG mediated process has been applied for the successful synthesis of various metal oxide nanomaterials of high uniformity.²⁴⁰⁻²⁴¹ In this work, EG could also assist the formation of more uniform and less agglomerated LDH nanoplatelets during the precipitation and aging process. Upon filtration/washing/drying, more oriented aggregation is resulted from the packing of these individual nanoplatelets in *c*-direction. In addition, it is found that the size of LDH nanoplatelets synthesized in EG and water mixture is larger than that in water alone. This could be due to the different solvation effects of EG and water, which in turn influence reaction rates.²⁴² However the exact role that EG plays requires more detail investigation.

In fact, the interest on particle-particle interactions in LDH materials has been raised recently for the purpose of generating LDH thin film for practical devices. Several groups have studied the interaction behavior of LDHs. Jun and co-workers produced oriented single and multi-layered LDH assembly on Si support by drying the suspension of LDH particles in organic solvent.^{87, 243-244} Wang et al. successfully developed a technique to fabricate the transparent and dense LDH films through the aggregation of uniformly sized LDH particles in *c*-orientation on various substrates.¹⁶⁸ Transparent LDH film on a glass slide was also produced by drying the aqueous colloidal LDH suspension obtained by dispersing LDHs prepared in methanol.¹⁶⁶⁻¹⁶⁷ Delamination of LDHs is another method to generate colloidal LDH particles which are then used as building blocks to fabricate oriented LDH films.^{162, 245-246} Based on all these studies, it is understood that face-to-face aggregation between individual LDH platelets is the main driving force to form continuous oriented film. Moreover, the prerequisite to induce face-to-face interaction is a stable colloidal suspension of individual LDH particles of preferably regular size and shape, from which the LDH platelets are deposited with their basal planes resting parallel to the substrate upon solvent evaporation.¹⁶⁸ Otherwise, edge-to-face interactions become significant and the resulting aggregates are made of randomly oriented LDH platelets with inter-particle porosity.^{166, 168}

Hydrothermal treatment was reported to be effective in obtaining a stable colloidal suspension of LDH particles. It was also found that an important step is to remove extra salts after complete precipitation and prior to hydrothermal treatment.^{244, 247} However, such strategy is usually applicable to the synthesis of LDHs with easily intercalated anions (e.g. CO_3^{2-}). The presence of Ibp in the mother liquor during the aging process is important to ensure a maximum extent of its intercalation in competing with the inorganic counter anions (NO_3^- in this case). In this work, it was found that a proper solvent system combined with the hydrothermal treatment results in a relatively more stable suspension for sample EG/W-H. Based on the TEM results, it is believed that ethylene glycol in the solvent reduces the effect of the salts for edge sharing, therefore, leads to less aggregation of LDH particles in suspension. In addition, regularly shaped and larger sized platelets in sample EG/W-H should also induce preferential aggregation by their largest faces during the post washing and drying processes. Although the formation of thin films is not the aim of this study, the interaction between drug loaded LDH particles is similarly the key factor leading to different aggregation behavior in the dry powders, which in turn affects the drug release profile from the dry powders. To the best of our knowledge, no such prior attention has been paid significantly by other researchers.

4.3.3 *In vitro* drug release test

The drug release profiles of Ibp from Mg_2Al -Ibp-LDH powders dispersed and continuously stirred in the buffer solution (pH 7.0) are displayed in Figure 4-7. The size of the ground particles used for the release study was between 20-80 mesh. Table 4-2 shows the detailed size distribution. The table shows that samples EG-W-H and W-H relatively have similar distribution, as well as those EG-W-C and W-C. Hence, different preparation method may lead to different aggregate sizes, despite the same post-synthesis treatment applied to all of the samples (washing and drying methods).

Table 4-2. The size distribution of the ground particles used for the drug release study.

Sample	% weight					
	20 mesh	30 mesh	40 mesh	50 mesh	60 mesh	≤ 80 mesh
EG-W-H	9.5	11.6	15.1	10.5	18.2	35.1
EG-W-C	0.4	3.0	5.0	5.1	10.8	75.7
W-H	10.1	14.1	19.0	11.4	17.5	27.8
W-C	2.8	3.0	6.7	5.9	15.6	66.0

While a complete release of Ibp is observed from all the samples and is also confirmed by FTIR spectra after release (Figure 4-9), the release rate depends on the aggregation state of the powder sample. The oriented powder sample, EG/W-H, exhibits a considerably slower release rate than the other three randomly aggregated samples, W-H, EG/W-C and W-C. It should be noted that sample W-H shows a similar drug release profile to those of EG-W-C and W-C (with the initial burst release) despite having larger powder size (Table 4-2), while sample EG-W-H shows slower release. In this case, the powder size may not play a main role in controlling the release rate, but rather, the morphology of the particles. The time required for 50% ($t_{50\%}$) and 90% ($t_{90\%}$) of release from sample EG/W-H is approximately 68 and 160 min respectively, which is several times longer compared with the other three samples (Table 4-1).

Figure 4-7. Release profiles of Ibp from the Mg_2Al -Ibp-LDH samples in phosphate buffer solution, initial pH = 7.0, initial total concentration of anions (OH^- and H_2PO_4^-) = 0.079 M.

A sustained drug release system shall allow an effective therapeutic blood concentration to be retained for a long period of time based on a reduced number of dosages applied to patients. This requires a slower release rate of drug molecules from the solid matrix. The release profiles of samples W-H, EG/W-C and W-C are not significantly different from each other and are also similar to that reported by Ambrogi et al. for a Mg_2Al -Ibp-LDH sample prepared by the anion-exchange route.¹⁹⁵ However, a much faster rate ($t_{90\%} = 13$ min) was observed by O'Hare and co-workers.¹⁰⁰ Nonetheless, it has to be pointed out that the absolute comparison among the release data from different research groups is impractical even for the drug-LDHs with similar compositions, due to different experiment conditions employed during the *in vitro* release test, such as the buffer composition, the mixing/stirring rate, etc. The initial rapid release or the burst effect appearing in samples W-H, EG/W-C and W-C is not significant for sample EG/W-H, which is an important aspect for

a decreased initial fluctuation of drug concentration and reduced toxicity for drugs causing various side effects.²⁴⁸

Figure 4-8. Drug-LDHs aggregation state in dry powder (a and b) and drug release model from LDHs in neutral buffer condition (c).

In the neutral buffer solution, the drug release process can be schematically represented by the model in Figure 4-8c. During this process, the inorganic anions (phosphate and hydroxyl anions) from the buffer solution are exchanged with the interlayer drug anions in the solid matrix. It is worth noting that dispersion of individual LDH platelets during the release test shall not be substantial, as the solid particles can easily settle at the bottom of the beaker when the stirring was stopped. The diffusion rates of the inorganic anions and drug anions in and out of the diffusion layer of LDH aggregates can be controlled by the characteristics of the powder, including the size of the aggregates, the porosity, the crystallinity of LDH platelets, etc. For the dense and oriented powder sample (EG/W-H, Figure 4-8a), the average diffusion path length is longer and the diffusion resistance is higher due to larger aggregates and the rigid structure. Furthermore, the formation of dense aggregates also results in low porosity so that the diffusion rate of the drug from the LDH

solid matrix is lowered. As a result, the release rate of Ibp from this sample is considerably lower than those from loosely aggregated powders, which are formed predominantly by the edge-to-face aggregation (Figure 4-8b).

The FTIR analysis results for the samples dried after drug release test are shown in Figure 4-9. The complete release of Ibp from all the samples is evidenced from the absence of characteristic absorption peaks for Ibp anion. Instead, the absorption band at around 1080 cm^{-1} is present due to the stretching vibration of phosphate anion.⁷⁸ The SEM images shown in Figure 4-10 indicate that overall, fractions of smaller aggregates are present in samples after release study, which could be due to the partial breakdown of aggregates by stirring during the release test. Nevertheless, sample EG/W-H still largely remains as dense aggregates and relatively less breakdown was observed (Figure 4-10a). Therefore, the more severe breakdown of aggregates along the release test in the other three samples should also be an important factor leading to the faster release rates.

Figure 4-9. FTIR spectra of dried samples after the *in vitro* release test of Mg₂Al-Ibp-LDH samples, (a) EG/W-H; (b) W-H; (c) EG/W-C; and (d) W-C.

Figure 4-10. SEM images of dried samples after the *in vitro* release test of $\text{Mg}_2\text{Al-Ibp-LDH}$ samples, (a) EG/W-H; (b) W-H; (c) EG/W-C; and (d) W-C.

4.4 Conclusion

It is clearly demonstrated that through a proper control of the aggregation state of the dry powder by varying particle-particle interactions, the drug release rate from the drug-LDH solid matrix can be tuned. We have prepared drug-LDHs with different morphological features which lead to different aggregation states among the corresponding dry powders and subsequently different drug release profiles, using ibuprofen as a model drug intercalated in $\text{Mg}_2\text{Al-LDHs}$. The sample obtained in the mixture of ethylene glycol and water under the hydrothermal condition forms *c*-oriented dense powder upon drying in oven without extra steps. This could be attributed to the preferential surface-to-surface and edge-to-edge aggregations of the large, regularly shaped and crystalline drug-LDH platelets. The use of ethylene glycol in the solvent system is found to reduce the aggregation of LDH particles in

the reaction mixture, which is an important factor for oriented attachment of LDH platelets. The release rate of ibuprofen is considerably slower from the oriented powder sample than that from the loosely aggregated powders. It is believed that such findings shall be able to extend to LDHs containing other drugs.”

CHAPTER 5 SYNTHESIS OF UNIQUE CORAL-LIKE LAYERED DOUBLE HYDROXIDE MICROSPHERES IN A MIXTURE OF NONAQUEOUS POLAR SOLVENT/SURFACTANT SYSTEMS

(This chapter is reproduced with permission from “Gunawan, P. and Xu, R., *Synthesis of Unusual Coral-like Layered Double Hydroxide Microspheres in a Nonaqueous Polar Solvent/Surfactant System*. Journal of Materials Chemistry 2008, 18, 2112-2120.” Copyright 2008, The Royal Society of Chemistry)²⁴⁹

5.1 Introduction

In the author's paper in *Journal of Materials Chemistry*,²⁴⁹ it is said “Advanced materials fabrication coupled with the control of morphological complexity and diversity is of great scientific and technological interest.” The interesting intercalation chemistry and compositional flexibility of LDHs have been greatly demonstrated with numerous types of constituting metal cations and interlayer anions by many researchers.^{232, 250} However, majority of these LDHs were synthesized in aqueous systems by a coprecipitation method and the resultant crystallite morphology is usually hexagonal platelets.^{120, 239, 244, 247} Upon drying, these platelets severely form irregular stone-like aggregates in the powder, which may affect their performances in many applications. Therefore, it would be advantageous to develop a method to prepare LDH particles with higher structural orders compared with the two-dimensional hexagonal platelets.

There have lately been some attempts to modify the structure and the morphology of LDHs. For example, one-dimensional LDHs with fiber, rod, or needle-like morphologies have been obtained by several groups, under the influence of organic solvents or hydrothermal conditions.^{152-153, 251-252} In particular, Hu and O'Hare reported the synthesis of belt-like Mg₂Al-LDHs with different orientations using reverse microemulsions which were

modified by triblock copolymers.¹⁵⁴ In another investigation, LDH nanospheres with diameters of *ca.* 200 nm were formed with their surface modified by polyoxyethylene sulfate during the anion-exchange process.¹⁵⁵ Li et al. synthesized Ni₂Al-LDH nanoplates with curved edges in chitosan solution.¹⁵¹ These results indicate that the utilization of unconventional synthesis media and the surface modifying agents might generate LDHs with new structures and morphologies that could not be obtained under normal co-precipitation conditions. The properties of the organic solvents, like the type of functional group and polarity, lead to different solvation effects which in turn influence the reaction rates.²⁴² In fact, an earlier work was reported on the effect of organic polar solvents on the textural properties of LDHs synthesized in a water-organic solvent mixture of a 1:1 volumetric ratio. The use of various organic solvents such as the short-chained alcohols, ethylene glycol, glycerol and acetone led to different surface areas and porosities of the plate-like LDH aggregates.²³⁰ Surfactants are also often utilized to assist the fabrication of well-defined inorganic nanocrystals with controlled size, shape and crystallinity.^{47, 253-255} Surfactant solution systems are rather complicated as the interactions among surfactant molecules and between the surfactant and the solvent are dependent on many factors. During the syntheses of inorganic nanocrystals, surfactants often play their roles as soft templates after forming micelles or microemulsions. In many cases, the individual surfactant molecules also function as surface capping agents that control the size, morphology and even the organization of the synthesized nanocrystals.

The surfactant aggregation in nonaqueous polar solvents has been a subject of increasing interest recently.²⁵⁶ Based on the investigations on many different combinations of surfactants and solvents, it is evident that surfactants aggregate in the form of micelles, liquid crystals or microemulsions in these polar solvent systems. Although the general aggregation behavior is similar to that in water, there are quantitative differences between the nonaqueous systems and aqueous systems. For example, the critical micelle concentration (CMC) of

surfactants is usually higher and the microemulsions formed in nonaqueous solvents often have a more disordered microstructure than those in water.²⁵⁷⁻²⁵⁸

In this chapter, we present the work on synthesis of dodecyl sulfate (DS)-intercalated Mg-Al LDHs in a mixture of nonaqueous polar solvents in the presence of DS anionic surfactant. It was found that porous coral-like LDH microspheres was obtained first time, although similar structured α -Ni(OH), CaCO_3 , MgO, $\text{Cu}(\text{OH})_2$ particles, etc. were reported by other groups.²⁵⁹⁻²⁶² The as-prepared LDH microspheres were further subjected to anion exchange with different types of drug compounds to access their suitability as drug reservoirs and carriers. These unique hierarchical LDH microspheres might be potentially used with many advantages over the conventional platelet aggregates.

5.2 Experimental Methods

5.2.1 Synthesis of dodecyl sulfate (DS) intercalated LDH

All reagents were of analytical grade and used without further purification. DS-intercalated MgAl-LDH was prepared using a precipitation method followed by solvothermal treatment. Briefly, 20 mL of a mixture of magnesium nitrate ($\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 99%, Acros) and aluminium nitrate ($\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, 98%, Acros) with a total concentration of 0.45 M and a $\text{Mg}^{2+} : \text{Al}^{3+}$ molar ratio of 2.0 was added drop wise at room temperature into 80 mL of vigorously stirred sodium hydroxide solution (NaOH, 99%, Merck) containing sodium dodecyl sulfate (SDS, 99%, Alfa Aesar). Both solutions were prepared in the mixture of ethylene glycol (EG) and methanol with a 1:1 volumetric ratio. The molar ratio of NaOH to the total metal cations (both Mg^{2+} and Al^{3+}) was 2.0 and the molar ratio of SDS to Al^{3+} was also 2.0. The salt feeding rate was kept around 0.66 mL min^{-1} and the stirring rate was 300 rpm. Before the addition of salt solution, the solution of NaOH and SDS mixture was bubbled with nitrogen (SOXAL, 99.999%) at 60 mL min^{-1} for 30 min to purge away carbon

dioxide from the synthesis chamber and the purging was continued during the addition of salt solution.

After the complete addition of salt solution, no precipitate was observed and the solution remained clear. The pH measured for this mixture using a well-calibrated pH meter was 9.0. The clear solution was then transferred into a Teflon-lined autoclave and aged at 150 °C for 18 h. After the aging, the pH of the mother liquor was measured as 7.6. The resultant precipitate was centrifuged and washed thoroughly with deionized water followed with ethanol. A fraction of the sample was then dried in the oven at 70 °C overnight for characterization. The remaining portion was kept wet in a closed bottle at room temperature for anion exchange with drugs. The above synthesis procedure was carried out under different experimental conditions to study the effect of several parameters, such as aging temperature, reaction time, and solvent type.

5.2.2 Anion exchange with drug compounds

A certain amount of wet sample of dodecyl sulfate (DS)-intercalated MgAl-LDH was dispersed in 20 mL of a mixture of EG and methanol with a 1:1 volumetric ratio followed by ultrasonication for 30 min. Another solution containing desired drug (NSAID), such as ibuprofen sodium salt (99%, Sigma-Aldrich), naproxen sodium salt (99%, Sigma-Aldrich), and 4-biphenylacetic acid (99%, Acros) was prepared by dissolving the drug in 80 mL of the same solvent mixture. The pH of drug solution was adjusted to 10.0 by adding NaOH solution (0.1 M). The drug solution was bubbled with nitrogen at 60 mL min⁻¹ for 30 min. The LDH suspension was then quickly added into the drug solution. The anion exchange was carried out in a closed flask with continuously stirring at room temperature for 24 h. The resulting gel was centrifuged and washed thoroughly with deionized water followed with ethanol, and then dried in the oven at 70 °C overnight.

5.2.3 Materials characterizations

The as-synthesized LDH samples and drug exchanged LDH samples were characterized using XRD, FTIR, BET, TGA, ICP, CHN, TEM, and SEM methods as described in Chapter 3. The weight percentage of various intercalated drugs was measured using UV-vis spectroscopy after a complete dissolution of the drug exchanged LDH samples in the mixture of 1.0 M HCl aqueous solution and ethanol of 1:1 volume ratio.

5.3 Results and Discussion

5.3.1 Structural, chemical, and morphological properties of DS-intercalated Mg-Al LDHs

The experimental conditions and sample names are summarized in Table 5-1. Briefly, the effects of solvent compositions, solvothermal temperature, and aging time on the LDHs structure and morphology are investigated. The solvent compositions were varied as pure methanol (sample D-EG0), pure ethylene glycol (sample D-EG100), and the mixture of both solvents with 1:1 volumetric ratio (samples A, B, and C). The aging temperature was varied at 100, 120, 150, and 180 °C, and the aging time was varied 4, 8, 18, and 48h.

Table 5-1. Experimental conditions for the prepared samples.

Sample	EG:MeOH vol ratio	Temperature (°C)	Aging time (h)	Morphology
A	1:1	150	18	Coral-like microsphere
B-100C	1:1	100	18	Rigid microsphere
B-120C	1:1	120	18	Rigid microsphere
B-180C	1:1	180	18	Irregular platelet
C-4h	1:1	150	4	Rigid microsphere
C-8h	1:1	150	8	Coral-like microsphere
C-48h	1:1	150	48	Coral-like microsphere
D-EG0	0:1	150	18	Irregular platelet
D-EG100	1:0	150	18	Irregular platelet

Figure 5-1. SEM (a-c) and TEM (d) images of DS-intercalated Mg-Al LDH (sample A) prepared in a mixture of EG and methanol of 1:1 volumetric ratio at 150 °C for 18 h.

Figure 5-1 displays the morphological features of sample A, which was synthesized in the mixture of EG and methanol of 1:1 volumetric ratio at 150 °C for 18 h. Distinct from the conventional stone-like aggregates, the overview SEM image in Figure 5-1a shows the formation of well-defined microspheres of about 0.5-1 μm in diameter. The higher magnification images in Figure 5-1b and 5-1c show that the microspheres have a coral-like hierarchical structure consisting of self-organized nanoplatelets. Such structure is also confirmed by the TEM images as shown in Figure 5-1d. The aggregation of thin and curved nanoplatelets can be clearly observed in the rim zone of the microsphere. The formed microspheres are not hollow as evidenced from Figure 5-1d inset. Broken parts were occasionally observed during our SEM analysis and the interior structure of the microspheres

was also found coral-like (Figure 5-2 (A)). As comparison, Figure 5-2 (B) presents the interior structure of the rigid microsphere. It can be noted that the constituent nanoparticles that compose the coral-like microspheres are larger and show distinct shape while those of the rigid microspheres are much smaller and are not well-defined. More detailed evolution mechanism from rigid microspheres to coral-like ones will be discussed in the later part of this chapter.

Figure 5-2. SEM images of the interior structures of (A) coral-like and (B) rigid microspheres, as observed from the broken spheres.

The formation of DS-intercalated MgAl-LDH for sample A and other samples is evident from their XRD patterns and FTIR spectra. As shown in Figure 5-3, the XRD patterns of all the samples synthesized in EG/methanol mixture have a rhombohedral structure with $a = b$ and $c = 3d_{003}$ unit cell. The basal peaks, apart from the first (003) peak, are all very weak, indicating the poor crystallinity of these samples. The d -spacing (d_{003}) of the coral-like microspheres is 32.4 Å, which is slightly higher than the previously reported values of about 26 Å.^{111, 138, 154, 263} Such a slight expansion could be due to different solvent systems that would further lead to different orientation of DS anion. In agreement with many other studies, it is suggested that a bilayer arrangement of DS anions exists in the interlayer space in accordance with the van der Waals axial length of DS of 20.3 Å.²⁶⁴ In addition to (003) peak, there is a broad band observed at 2θ around 20° , which is absent in either nitrate or sulfate-

intercalated LDHs. This band is attributed to the scattering of the X-ray by the carbon chain of DS.²⁶⁵

Figure 5-3. XRD patterns of DS-intercalated Mg-Al LDH samples prepared in a mixture of EG and methanol of 1:1 volumetric ratio at different temperatures and aging time, (a) sample A, 150 °C, 18 h; (b) sample C-8h, 150 °C, 8h; (c) sample C-4h, 150 °C, 4 h; (d) sample B-120C, 120 °C, 18 h; and (e) sample B-100C, 100 °C, 18 h.

FTIR spectra of all those samples are shown in Figure 5-4 and display bands that correspond to DS anion and the host LDHs. The broad band at 3400-3500 cm^{-1} corresponds to O—H stretching of the hydroxide layers. A shoulder at 1635 cm^{-1} is attributed to the bending mode of the adsorbed H_2O and the peak at 440-450 cm^{-1} is due to M—O lattice vibration of the hydroxide layers. The presence of DS anion is indicated by the strong absorption peaks at 1066 cm^{-1} and 1216 cm^{-1} that are assigned to the asymmetric and symmetric S=O stretching in the head group, respectively.²⁶⁶ The existence of C—H stretching and bending modes of DS alkyl chain correspond to the bands at 2850-2960 cm^{-1}

and 1469 cm^{-1} , respectively.²⁶⁶⁻²⁶⁸ However, the spectrum of sample A (Figure 5-4e) shows a broadening at around 1110 cm^{-1} which indicates a trace presence of free sulfate ion. Such an observation is not obvious for other samples synthesized at either lower temperature (100 and $120\text{ }^{\circ}\text{C}$) or shorter aging time (4 and 8 h). It could be due to partial decomposition of DS anion. Meanwhile, the characteristic absorbance of the counter nitrate anion at 1384 cm^{-1} is not observed, indicating that this anion is not intercalated, despite its abundant amount in the solution. This could be due to a more electronegative sulfate polar head present in DS anion, compared to nitrate ion, so that DS anion has stronger affinity to LDH layers.

Figure 5-4. FTIR spectra of DS-intercalated Mg-Al LDH samples prepared in a mixture of EG and methanol of 1:1 volumetric ratio at different temperatures and aging time, (a) sample B-100C, $100\text{ }^{\circ}\text{C}$, 18 h; (b) sample B-120C, $120\text{ }^{\circ}\text{C}$, 18 h; (c) sample C-4h, $150\text{ }^{\circ}\text{C}$, 4 h; (d) sample C-8h, $150\text{ }^{\circ}\text{C}$, 8h; and (e) sample A, $150\text{ }^{\circ}\text{C}$, 18 h.

The effects of temperature and aging time on the morphologies of DS-intercalated Mg-Al LDH can be observed from the SEM images of samples B-100C, B-120C, C-4h and C-8h in Figure 5-5. Sample B-100C obtained at a lower temperature of 100 °C while with other conditions identical to those for sample A exhibits rigid microspheres (Figure 5-5a). The microsphere is observed with a smooth edge in the TEM image (Figure 5-5a inset). However, it can be seen from the high-resolution SEM image that these rigid microspheres are formed by the aggregation of nanoparticles, which gives rise to rough surfaces. When a higher synthesis temperature (120 °C) was used, the sizes of the constituent nanoparticles became larger, as shown in Figure 5-5b for sample B-120C. Considering the morphological changes in samples B-100C, B-120C and sample A, it is believed that a higher synthesis temperature promotes the continuous growth of constituent nanoparticles into nanoplatelets through recrystallization in the microspheres, which eventually leads to a coral-like porous structure at 150 °C. Such a formation route is further confirmed from the time-dependent experiments performed at 150 °C. At 4 h, the LDH precipitate (C-4h) is already in the form of rigid microspheres of nanoparticles (Figure 5-5c). When the aging time was increased to 8 h, the coral-like structure started to appear by the observation of self-organized nanoplatelets on the surface of sample C-8h (Figure 5-5d). The estimated crystallite sizes in *c* direction based on the FWHM (full width at half-maximum) of (003) peak by Scherrer formula is 72, 84 and 101 Å for samples C-4h, C-8h and sample A (18 h), respectively, which correspond to 3-4 monolayers. For comparison, the BET specific surface area of the rigid microsphere (sample B-100C) and coral-like microsphere (sample A) are measured as 3.3 and 47.5 m²/g, respectively, confirming that the porous coral-like structure gives higher surface area.

Figure 5-5. SEM images of DS-intercalated Mg-Al LDH samples prepared in a mixture of EG and methanol of 1:1 volumetric ratio at different temperature and aging time, (a) sample B-100C, 100 °C, 18 h; (b) sample B-120C, 120 °C, 18 h; (c) sample C-4h, 150 °C, 4 h; and (d) sample C-8h, 150 °C, 8h.

In the other two experiments, samples B-180C and C-48h were synthesized at a higher temperature (180 °C, 18 h) and a longer aging time (150 °C, 48 h) respectively. With a longer aging time (sample C-48h, Figure 5-6a), the constituent nanoplatelets of the coral-like microspheres become larger due to continued growth, while leading to partially collapsed or irregularly shaped microspheres. On the other hand, sample B-180C (Figure 5-6b) consists of irregular aggregates of platelet-like particles. Further analyses of the crystal structure and chemical bonding information reveal that these two samples are not intercalated with DS anions. Their basal spacing (d_{003}) obtained from XRD (Figure 5-7) is reduced to be about 9.3 Å, which agrees with that of sulfate-intercalated LDHs produced by Gillman, et al.²⁶⁹ (9.58 Å) and Constantino, et al.¹²¹ (8.9 Å). FTIR results (Figure 5-8) also show that the characteristic absorption peaks of DS are greatly reduced while the strong absorbance at about 1110 and

618 cm^{-1} that are attributed to the stretching of free sulfate anions (ν_3 and ν_4 mode) appear.²⁷⁰⁻

²⁷¹ In addition, a second phase of a basic salt, magnesium basic sulfate, $\text{Mg}_{1.33}(\text{SO}_4)(\text{OH})_{0.66} \cdot 0.33\text{H}_2\text{O}$ (JCPDS # 037-0097, marked with asterisks in Figure 5-7b), is also present in sample B-180C. These observations confirm that free sulfate anions were produced under these experimental conditions. Previous studies suggested that hydrolysis of DS takes place particularly when the temperature is high (reaction I).²⁷² In an alkaline condition, sulfate is produced by reaction II.

For the synthesis of sample C-48h, the above reactions probably occurred after the formation the coral-like microspheres during the prolonged aging. As a result, the coral-like structure is not severely affected by the transformation of DS (Figure 5-6a). However, at a higher temperature such as the synthesis of sample B-180C, reaction (I) proceeded at a faster rate. The generated free sulfate anions were readily intercalated in Mg-Al LDH or reacted with metal cations and OH^- to form the basic salt (magnesium sulfate hydroxide). Due to the occurrence of such reactions, normal crystallites of platelet morphology were formed, which did not self-assemble to microspheres.

Figure 5-6. SEM images of samples prepared in a mixture of EG and methanol of 1:1 volumetric ratio at different temperature and aging time, (a) sample C-48h, 150 °C, 48 h; and (b) sample B-180C, 180 °C, 18 h.

Figure 5-7. XRD patterns of samples prepared in a mixture of EG and methanol of 1:1 volumetric ratio at different temperature and aging time, (a) sample C-48h, 150 °C, 48 h; and (b) sample B-180C, 180 °C, 18 h, * indicates $\text{Mg}_{1.33}(\text{SO}_4)(\text{OH})_{0.66} \cdot 0.33\text{H}_2\text{O}$ phase (JCPDS # 037-0097).

Figure 5-8. FTIR spectra of samples prepared in a mixture of EG and methanol of 1:1 volumetric ratio at different temperature and aging time, (a) sample C-48h, 150 °C, 48 h; and (b) sample B-180C, 180 °C, 18 h.

The thermal behavior of the samples prepared with different aging temperature and aging time is shown by the TGA and DTA curves in Figures 5-9 to 5-12. The samples were heated up to 1100 °C in a flowing air. The weight loss from room temperature up to 200 °C is observed for all samples, which is due to evaporation of the intercalated and the adsorbed water molecules. Subsequently, samples A, B-100C, B-120C, C-4h, and C-8h (Figures 5-9 a-c and 5-11 a-c) exhibit great weight loss at around 280 °C due to combustion of DS carbon chain, which corresponds to a sharp exothermic peak in their corresponding DTA curves (Figures 5-10 a-c and 5-12 a-c). Afterwards, there is a continuous weight loss up to around 400 °C due to dehydroxylation process of LDH layers, which is indicated by a weak and broad shoulder in DTA curves. Further combustion of the remaining carbons (those attached

to sulfate group of DS) occurs at around 640 °C, as evidenced by the presence of an exothermic hump on DTA curves. In addition, samples B-100C and C-4h display another small weight loss at around 720-740 °C, which could be due to partial decomposition of the sulfate group. For the remaining DS-intercalated LDH samples, this process starts after the complete removal of the hydrocarbon species at higher temperatures, around 950-1000 °C.²⁶⁷ The early decomposition of the sulfate group for samples B-100C and C-4h could be due to a loose attachment of DS anion in the interlayer as LDH platelets were not well developed. Thermal decomposition process of samples B-180C (Figures 5-9d, 5-10d) and C-48h (Figures 5-11d, 5-12d) is similar, except for the sulfate decomposition process. In general, dehydroxylation of LDH layers takes place at about 420 °C for both samples. The subsequent weight loss at about 670 °C is due to decomposition of the intercalated sulfate anion in LDH interlayer.¹²¹ Different from sample C-48h, which is pure LDH intercalated mostly with sulfate anion, samples B-180C contains magnesium sulfate hydroxide (see XRD result, Figure 5-7). As a result, the relatively intense DTA peak for sample B-180C at about 1000 °C (Figure 5-10d) can be ascribed to decomposition of sulfate in this magnesium salt which is significantly present in the sample.

Figure 5-9. TGA curves of samples prepared at varied aging temperature, (a) 100 °C (sample B-100C), (b) 120 °C (sample B-120C), (c) 150 °C (sample A), and (d) 180 °C (sample B-180C), with aging time 18 h. The numbers in the brackets are the residue percentages.

Figure 5-10. DTA curves of samples prepared at varied aging temperature, (a) 100 °C (sample B-100C), (b) 120 °C (sample B-120C), (c) 150 °C (sample A), and (d) 180 °C (sample B-180C), with aging time 18 h.

Figure 5-11. TGA curves of samples prepared at 150 °C with varied aging time, (a) 4 h (sample C-4h), (b) 8 h (sample C-8h), (c) 18 h (sample A), and (d) 48 h (sample C-48h). The numbers in the brackets are the residue percentages.

Figure 5-12. DTA curves of samples prepared at 150 °C with varied aging time, (a) 4 h (sample C-4h), (b) 8 h (sample C-8h), (c) 18 h (sample A), and (d) 48 h (sample C-48h).

The compositions of the DS-containing LDH microspheres are summarized in Table 5-2. The elemental analysis gives a Mg:Al molar ratio in a narrow range of 1.98 to 2.06, which is approximately the ratio in the metal precursor solution. Nitrogen was not detected by CHNS elemental analysis, indicating the absence of both intercalated and adsorbed nitrate from the precursor solution. The overall chemical formulae shown in Table 5-2 suggest that DS is the main interlayer anion in all the samples. However, certain percentages of carbonate could be co-present in the interlayer space based on our compositional analysis, especially for samples obtained at a higher temperature and longer aging duration (samples A and B-120C). In addition, samples A and C-8 h may also intercalate small amounts of sulfate as discussed earlier. The accuracy of the determined formulae is acceptable by comparing the measured oxide residue percentages (by TGA) against the calculated values based on the formulae. In

agreement with other studies, the results shown in Table 5-2 also indicate that there is a direct correlation between the aging time and temperature and the amount of intercalated DS. With the increase of aging temperature and time, the molar ratio of DS to Al^{3+} decreases. At the same time, the amount of free sulfate ions increases as a result of partial decomposition of DS at a higher temperature and prolonged aging time.

Table 5-2. Chemical compositions of the prepared microspheres DS-containing Mg-Al LDHs.

Sample	%Mg ^a (wt%)	%Al ^a (wt%)	%C ^b (wt%)	%S ^b (wt%)	%H ₂ O ^c (wt%)	Chemical formula	% Residue	
							TGA ^d	Calc. ^e
A	11.92	6.69	23.42	5.30	8.71	Mg _{1.98} Al(OH) _{5.96} (DS) _{0.62} (SO ₄) _{0.02} (CO ₃) _{0.17} .1.96H ₂ O	34.90	33.60
B-100C	10.81	5.84	27.96	6.03	11.68	Mg _{2.05} Al(OH) _{6.12} (DS) _{0.88} (CO ₃) _{0.06} .3.00H ₂ O	30.29	28.43
B-120C	11.59	6.31	23.98	5.09	10.98	Mg _{2.04} Al(OH) _{6.08} (DS) _{0.68} (CO ₃) _{0.16} .2.56H ₂ O	33.78	32.14
C-4h	10.66	5.94	27.41	5.85	11.25	Mg _{1.99} Al(OH) _{5.98} (DS) _{0.87} (CO ₃) _{0.06} .2.83H ₂ O	30.68	28.39
C-8h	11.30	6.25	25.05	5.70	9.56	Mg _{2.01} Al(OH) _{6.01} (DS) _{0.72} (SO ₄) _{0.03} (CO ₃) _{0.11} .2.29H ₂ O	32.28	31.43

^a The weight percentages of Mg²⁺ and Al³⁺ are obtained from ICP measurement.

^b The weight percentages of C and S are obtained from CHNS elemental analysis.

^c The weight loss percentage of dry sample by TGA analysis from 25 °C to 200 °C.

^d The weight percentage of mixed Mg-Al oxide residues from TGA analysis.

^e The weight percentage of mixed Mg-Al oxide residues calculated based on the chemical formula.

5.3.2 Proposed mechanism for the formation of the coral-like microspheres LDHs

Samples synthesized with the same experimental parameters but in other solvent systems such as water, methanol alone, or EG alone, all give the common platelet-like morphology. The unique physical and chemical properties associated with the current system of EG/methanol/DS mixture are found important for the formation of coral-like microspheres. The presence of DS is also essential for the formation of LDHs of microspherical structure as the sample prepared in the absence of DS gave normal LDH platelets. In alcohol solution, salts can be solvated just as in water in the form of either contact ion pair, salt-(solvent)_n for a simple salt like NaCl, or solvent-separated ion pair, cation-(solvent)_m···anion-(solvent)_p for more complicated salts.²⁷³⁻²⁷⁴ In this work, all the starting compounds, Mg(NO₃)₂·6H₂O, Al(NO₃)₃·9H₂O, NaOH and SDS were completely dissolved in the mixture of EG and methanol of 1:1 volumetric ratio. The influence of crystal water from the metal salt precursors shall not be significant since it is only present at a small quantity compared to the main solvents. The formation of solvated clusters of Mg²⁺, Al³⁺ and OH⁻ ions inside the cages of EG or methanol is well expected (Scheme 5-1). The counter anions were not shown in the scheme for clarity. In addition, EG is known as a polyol with strong complexing ability. In many studies, EG serves as a bidentate ligand to form coordination complexes with various metal ions, such as Ti⁴⁺, Sn²⁺, In³⁺, Pb²⁺, and Ni²⁺, etc.^{241, 275} In other cases, metal alkoxides are formed by alcoholysis of EG and coordination with metal ion centers, such as Co²⁺,²⁷⁶⁻²⁷⁷ Fe³⁺,²⁷⁸ etc. Although the coordination of EG with Mg²⁺ and Al³⁺ cations is seldom reported, the formation of [Mg(EG)₃]²⁺ and [Al(EG)₃]³⁺ complexes cannot be ruled out. In fact, the role of EG is critical as observed from the sample prepared in a control experiment without EG. Overall, the nucleation and crystallization processes of LDHs are effectively retarded in the present study due to the reduced activities of ions interacting with methanol and EG.²⁷⁹ As a result, it is important to note that even upon the complete addition of the metal precursor (Scheme 5-1a) into the alkaline solution (Scheme 5-1b), the resultant mixture was still in the

form of a clear solution. A photo of this mixture was taken against a dark background and is shown in Scheme 5-1c'. During the drop wise addition of metal precursor solution, continuous stirring was applied to ensure a uniform mixture at all time.

Scheme 5-1. Schematic illustration of the proposed formation route of coral-like LDH microspheres.

On the other hand, the concentration of the anionic surfactant DS in the alkaline solution was 75 mM. The CMC of DS in water is only about 8.2 mM. In organic polar solvents, the value increases slightly but shall not change drastically.²⁵⁷ Similar to the behavior in water, as the concentration increases, DS anions aggregate to liquid crystals of various shapes, and a lamellar phase can be formed when the concentration is much higher than the CMC.²⁵⁸ On such a basis, it is likely that DS anions form a bilayer lamellar phase in the alkaline solution with their polar head groups exposed to the solvents (Scheme 5-1b). The positively charged brucite-like sheets, once formed, should be easily attracted by the negatively charged DS head groups via electrostatic interaction (Scheme 5-1c). DS anions are thus incorporated as interlayer anions if both sides of the bilayer are attached with brucite-like sheets. Furthermore, considering the roles that surfactants play in many syntheses, we propose that DS anions also act as the surface modifying species of the formed LDH

nanocrystals (Scheme 5-1d where the intercalated DS anions are not shown). The conventional nonhomogeneous precipitation of LDHs in aqueous solution occurs immediately upon the mixing of metal cations and alkalis in the localized regions, which usually results in polydispersed particle sizes. In contrast, uniform nanocrystals were formed upon nucleation and crystallization in this work due to i) a homogeneous starting mixture of metal cations and alkali in the methanol/EG solvent, ii) the presence of DS as surface modifying species and iii) EG in the solvent serving as dispersant and stabilizer. Therefore, the growth of nanocrystals into larger platelets is prohibited. This was evidenced from the cloudy mixture (photo in Scheme 5-1c'') instead of bulk precipitates even at 30 min after the complete addition of metal precursor solution at room temperature. The nanocrystals formed further self-assemble into microspheres (Scheme 5-1e, resembling the images in Figure 5-5a and 5-5c) in order to minimize the surface energy during solvothermal treatment. At a sufficiently high temperature (150 °C) and long aging time (18 h), the coral-like hierarchical structure is formed as a result of recrystallization of the constituent nanoparticles in the microspheres to the thin nanoplatelets, which are the most stable crystal habitat of LDHs (Scheme 5-1f, resembling the images in Figure 5-1).

Figure 5-13. SEM images of Mg-Al LDH samples prepared in different solvent systems at 150 °C and 18 h, (a) sample D-EG0, synthesized in methanol alone; and (b) sample D-EG100 synthesized in ethylene glycol alone.

The SEM images of samples prepared in a single solvent system of methanol or EG are shown in Figure 5-13. They consist of irregular aggregates of platelet-like particles. Sample D-EG0 obtained in methanol alone gives dense aggregates (Figure 5-13a), while sample D-EG100 synthesized in EG alone exhibits aggregates of more porous structures (Figure 5-13b). In fact, precipitation occurred concomitantly with the addition of metal precursor solution into the alkaline solution for these two cases. The solvation effects by both EG and methanol as discussed earlier are crucial for the reduced nucleation and crystallization rates.

5.3.3 Anion exchange of DS intercalated Mg-Al LDHs with drug compounds

Recently, there has been increasing interest in the intercalation of different types of drug anions into LDHs. One of the main advantages of using such a material system lies in its high drug loading, since drugs are intercalated into the interlayer space at the molecular level, rather than being adsorbed merely on the surface of the particles. Besides, there are other incentives such as enhanced drug stability, improved drug solubility, sustained drug release, and improved efficacy of drugs.^{100-101, 195, 200, 206, 258} However, the morphological properties of the drug-LDHs in previous studies have not received any particular attention so far. It is believed that the unusual LDH microspheres produced in the present work might be used more advantageously over the conventional irregular aggregates, such as: (1) surface coating/functionalization with polymer, such as enteric polymers, will be more effective, because platelet-like particles aggregate severely, as reported by Li et al.,²⁰² (2) development of composites of LDH with other nanoparticles such as magnetic nanoparticles, luminescence substances, or silver nanoparticles as antimicrobial agent to render multifunctionality, by incorporating them inside the “pores” of the microspheres.

For instance, with the more defined morphology, the surface of these microspheres can be readily functionalized with polymers, or other coatings for more versatile applications.

In addition, a low degree of agglomeration and narrow sized distribution of these particles are desirable for the formation of thin films for broader applicability. With this regard, we investigated the anion-exchange capacity of DS-intercalated Mg-Al LDH microspheres with various drug anions, using both the rigid microspheres (sample B-100C) and coral-like microspheres (Sample A). The anionic forms of a cardiovascular drug, 4-biphenylacetic acid, and non-steroidal anti-inflammatory drugs, naproxen and ibuprofen as sodium salts, were used for the anion-exchange.

Table 5-3. Crystallographic parameters of drug-LDH microspheres.

Sample name	d_{003}^a (Å)	d_{110}^a (Å)	Gallery height ^b (Å)	a^c (Å)	Dp_{003}^d (Å)	No. of LDH layers ^e
A	32.4	1.52	27.6	3.04	101.5	3.13
B-100C	31.5	1.52	26.7	3.03	77.2	2.45
A/BPA	24.6	1.52	19.8	3.04	70.7	2.87
B-100C/BPA	25.0	1.51	20.2	3.02	67.8	2.71
A/Nap	23.0	1.52	18.2	3.04	81.1	3.53
B-100C/Nap	23.9	1.51	19.1	3.03	62.4	2.61
A/Ibp	23.2	1.52	18.4	3.03	68.9	2.97
B-100C/Ibp	25.4	1.51	20.6	3.02	67.1	2.64

^a Basal spacing d_{003} was calculated using (003) peak and d_{110} using (110) peak from the XRD pattern.

^b Gallery height is calculated by subtracting d_{003} with the thickness of hydroxyl layer (4.8 Å).

^c The inter-cation distance in the hydroxyl layer, $a = 2d_{110}$.

^d Crystallite size in c direction was calculated using Scherrer formula.

^e The number of layers was obtained by dividing Dp_{003} with basal spacing d_{003} .

The successful exchange of DS anions by drug anions is evidenced from the XRD and FTIR results shown in Figure 5-14 and Figure 5-15, respectively. The spectra of the pristine DS-intercalated Mg-Al LDHs (Figure 5-14 a and b, 5-15 a and b) are displayed together with the drug-exchanged samples (Figure 5-14 c-h and 5-15 c-h) for a better comparison. In Figure 5-14, a shift of (003) basal peak to a higher diffraction angle is observed for all drug-exchanged LDH samples due to the smaller axial length of the drug anions than that of DS. The information on the crystallographic parameters is presented in Table 5-3. It is noted that

the basal spacings of the samples after anion exchange are overall larger than those obtained by other authors while using the same Mg : Al molar ratio of 2 and anion exchange method.^{100-101, 195}

Figure 5-14. XRD patterns of (a) sample B-100C (the pristine DS-intercalated LDH rigid microsphere); (b) sample A (the pristine DS-intercalated LDH coral-like microsphere); (c) sample B-100C exchanged with 4-biphenylacetate (B-100C/BPA); (d) sample A exchanged with 4-biphenylacetate (A/BPA); (e) sample B-100C exchanged with anionic naproxen (B-100C/Nap); (f) sample A exchanged with anionic naproxen (A/Nap); (g) sample B-100C exchanged with anionic ibuprofen (B-100C/Ibp); and (h) sample A exchanged with anionic ibuprofen (A/Ibp).

All FTIR spectra of drug-exchanged samples give the characteristic peaks of the carboxylate group at ca. 1553 and 1396 cm^{-1} due to ν_{as} (antisymmetric) and ν_{s} (symmetric) stretching vibration modes, which is present in all these drug anions. At the same time, the strong peaks of DS anion in the region of 1066-1216 cm^{-1} are either disappeared or become

much less prominent and overlapped with some weak absorption peaks of drug anions in this region.

Table 5-4. Chemical compositions of drug-exchanged microsphere LDHs.

Sample	%Mg ^a (wt%)	%Al ^a (wt%)	%C ^b (wt%)	%S ^b (wt%)	%Drug ^c (wt%)	Mg : Al (molar)	S : Al (molar)	Drug : Al (molar)
A-Ibp	12.64	6.88	28.63	1.81	34.21	2.04	0.22	0.65
A-Nap	13.14	6.86	26.35	1.86	31.76	2.12	0.23	0.55
A-BPA	12.69	6.47	29.78	1.58	30.10	2.17	0.21	0.59
B-100C-Ibp	10.93	6.24	31.42	2.36	33.30	1.96	0.32	0.70
B-100C-Nap	11.02	6.06	31.40	1.79	33.73	2.01	0.25	0.66
B-100C-BPA	12.86	7.00	31.66	0.84	31.62	2.03	0.10	0.58

^a The weight percentages of Mg²⁺ and Al³⁺ are obtained from ICP measurement.

^b The weight percentages of C and S are obtained from CHNS elemental analysis.

^c The weight percentages of drug compound are obtained from UV-Vis spectroscopy.

The compositions of samples after anion exchange with drugs are shown in Table 5-4. The weight percentages of drug range 30–34% based on the UV-vis spectroscopy analysis. It is evidenced from the results that a complete anion exchange of the pristine DS and other inorganic anions by drug anions did not occur under the conditions of the present experiment. The sulfur detected should come from the remaining DS and/or sulfate in the compounds. Therefore, drug anions and other anions including DS, sulfate and carbonate are possibly co-present in these LDHs. As a result, the chemical formulae were not determined due to such complexity.

There are several publications regarding the intercalation of ibuprofen and naproxen into MgAl-LDHs with a molar Mg:Al ratio of 2. Ambrogi et al. reported the intercalation of ibuprofen by anion exchange at 60 °C.¹⁹⁵ Complete exchange was achieved within 3 days and it gave 50% drug loading. Del Arco et al. prepared Naproxen-LDHs by the coprecipitation method.¹⁰¹ The drug intercalation was incomplete with a drug : Al molar ratio of about 0.7-0.8. In another study, Wei et al. obtained 35% drug loading after 39 h of aging at 70 °C.⁴ For

the intercalation of biphenylacetic acid, the work done by Khan et al. using LiAl-LDH resulted in a drug loading of about 38%.¹⁰⁰ It is necessary to note that all of these studies were carried out in an aqueous system while in our case nonaqueous polar solvents (ethylene glycol/methanol) were used in order to prevent the change of morphology and to maintain the dispersion of the microspheres. As drug molecules usually have higher affinity to organic solvents, therefore they display less interaction with LDH layers. In addition, by comparing the coral-like microspheres and the rigid ones, the former contain more sulfate and carbonate anions that are originally in the interlayer. These anions have high affinity to the brucite-like layers and are difficult to be exchanged out. As a result, the drug loading is affected. Overall, the drug loadings in our MgAl-LDH microspheres are comparable with those in conventional LDHs reported by others. A complete exchange could occur in our case if the experimental conditions, such as temperature and duration, are further varied.

The SEM images of drug-intercalated LDH microspheres obtained via anion-exchange method from the pristine DS-intercalated LDH microspheres are displayed in Figure 5-16. The overall morphologies and the particle sizes are well retained after anion exchange experiments. The synthesis and anion exchange route developed in this work are simple and a high yield of drug-LDHs can be obtained. The process can also be easily scaled up for the fabrication of larger amounts of drug-LDHs for practical applications.

Figure 5-15. FTIR spectra of (a) sample B-100C (the pristine DS-intercalated LDH rigid microsphere); (b) sample A (the pristine DS-intercalated LDH coral-like microsphere); (c) sample B-100C exchanged with 4-biphenylacetate (B-100C/BPA); (d) sample A exchanged with 4-biphenylacetate (A/BPA); (e) sample B-100C exchanged with anionic naproxen (B-100C/Nap); (f) sample A exchanged with anionic naproxen (A/Nap); (g) sample B-100C exchanged with anionic ibuprofen (B-100C/Ibp); and (h) sample A exchanged with anionic ibuprofen (A/Ibp).

Figure 5-16. SEM images of (a) sample B-100C exchanged with 4-biphenylacetate (B-100C/BPA); (b) sample A exchanged with 4-biphenylacetate (A/BPA); (c) sample B-100C exchanged with naproxen (B-100C/Nap); (d) sample A exchanged with naproxen (A/Nap); (e) sample B-100C exchanged with ibuprofen (B-100C/Ibp); (f) sample A exchanged with ibuprofen (A/Ibp).

5.4 Conclusion

In summary, coral-like Mg-Al LDH microspheres can be fabricated via a simple and effective solvothermal method in a nonaqueous polar solvent/surfactant system of EG/methanol/DS. It was found that the rates of nucleation and crystallization of LDHs were effectively reduced in EG/methanol solvent mixture of 1:1 volumetric ratio due to the

solvation effects. The resultant LDH nanoparticles did not grow into the large platelets even at solvothermal temperatures of 100 °C and above. The presence of surfactant DS was found crucial in prohibiting such growth by modifying the surface of the nanoparticles. Through a self-assembly process, LDH microspheres consisting of the constituent nanoparticles can be formed at 100 °C. At a higher temperature of 150 °C, the constituent nanoparticles recrystallized to thin nanoplatelets of 3-4 monolayers, thereby resulting in a coral-like hierarchical structure. The as-formed DS-intercalated Mg-Al LDH microspheres of both rigid and coral-like structures were subjected to ion exchange with drug anions, such as 4-biphenylacetate, ibuprofen and naproxen anions. The anion-exchange was effective and the morphologies of the microspheres were well retained. It is believed that these LDH microspheres shall be found with wider applications compared to the conventional irregular aggregates of platelet-like LDHs.”

CHAPTER 6 DIRECT ASSEMBLY OF ANISOTROPIC LAYERED DOUBLE HYDROXIDE (LDH) NANOCRYSTALS ON SPHERICAL TEMPLATE FOR FABRICATION OF DRUG-LDH HOLLOW NANOSPHERES

(This chapter is reproduced with permission from “Gunawan, P. and Xu, R., *Direct Assembly of Anisotropic Layered Double Hydroxide (LDH) Nanocrystals on Spherical Template for Fabrication of Drug-LDH Hollow Nanospheres*. Chemistry of Materials 2009, 21, 781-783.” Copyright 2009, The American Chemical Society)²⁸⁰

6.1 Introduction

In the author’s paper in Chemistry of Materials,²⁸⁰ it is said “It is expected that fabrication of advanced functional materials with controlled 2D or 3D nanostructures would enhance their possibilities for practical applications. Up to date, the syntheses of LDHs with ordered structures have been reported by some research groups. For example, Geraud et al applied an “inverse opal” method to fabricate 3D macroporous LDHs, using polystyrene bead arrays as the sacrificial template.²⁸¹⁻²⁸² Nanosized LDHs were also formed inside the mesoporous carbon with large pores.²⁸³ In addition, growth or assembly of LDH nanocrystals on flat solid surfaces for generation of continuous, noncontinuous or patterned films has been achieved by several groups.^{162, 168, 243, 246, 284} However, direct assembly of LDH nanocrystals on surfaces of nano- or micro-sized spherical templates to fabricate core-shell type composites and LDH hollow spheres has not been reported, although Li et al constructed LDH nanoshells using the exfoliated LDH nanosheets by a layer-by-layer (LBL) process.¹⁶⁴ In fact, the LBL process via electrostatic interaction of oppositely charged layers has been frequently used for the assembly of preformed nanoparticles²⁸⁵⁻²⁹⁰ or exfoliated nanosheets^{287, 291-294} of various

materials, such as TiO_2 , SiO_2 , SnO_2 , Au, clay, $\alpha\text{-ZrP}$, niobate, etc., on spherical templates. In such a multi-step process, polyelectrolytes are often required as binders in each step. In addition, to preserve the spherical shape with a structural integrity during the subsequent thermal treatment, multilayer coating and post-sealing are necessary.^{286, 289, 294} Despite the frequent use of LBL method for production of core-shell composites or hollow materials, the multi-step process is tedious and time consuming. In this regard, a one-step and direct deposition method will be of great advantage.

Nanomaterials with hollow structures have attracted much attention in recent years owing to their unique properties, such as high surface area, good permeability, light weight, and peculiar optical/electrical/magnetic properties.²⁹⁵⁻²⁹⁷ Various types of sacrificial templates have been used, such as oil/water microemulsion, surfactant/polymeric micelles,^{52, 298-299} spherical silica,³⁰⁰⁻³⁰² polymers,³⁰³⁻³⁰⁴ and carbonaceous spheres.³⁰⁵⁻³⁰⁸ Among the hard template materials, carbon spheres have received much interest recently. The surface of carbon spheres is rich in hydroxyl (OH) and carbonyl (CHO) groups, thus providing active sites for adsorption or growth of functional materials.³⁰⁷ The carbon core can be easily removed by thermal decomposition for the generation of hollow spheres. In addition, unlike the preparation of polymeric materials, the fabrication of carbon nano- or microspheres is regarded environmentally friendly. The precursor used is simply sugar/polysaccharides dissolved in water and no organic solvents or harmful reagents are required.

In this chapter, we report a simple method for generating LDH hollow nanospheres via direct assembly of preformed anisotropic LDH nanocrystals on the surface of carbon nanospheres. Closely packed $\text{Mg}_2\text{Al-LDH}$ films were first formed on the carbon nanosphere template in one step. After removing the core by calcination, hollow nanospheres of MgAl-oxides with robust shell walls were formed. Further, the oxide shell can be readily converted to LDHs intercalated with functional anions (e.g., drug anions) based on the well-known memory effect.⁶² The major steps involved in this work are illustrated in Figure 6-1.

Figure 6-1. A scheme of one-step direct assembly process of nanocrystals towards the formation of LDH film on the surface of a spherical template and subsequent generation of hollow nanospheres of LDH intercalated with drug anions.

6.2. Experimental Method

6.2.1 Preparation of LDH-NCs and the control-LDH sample

Well-dispersed nanocrystals of LDH (LDH-NCs) was synthesized in pure methanol solvent (Fisher Scientific, 99.9%). Briefly, 20 mL of 0.45 M mixed solution containing $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.3 M, Acros, 99%) and $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (0.15 M, Aldrich, 98%), was added dropwise into 80 mL of vigorously stirred NaOH (0.225 M, Normapur, 99%) solution of the same methanol solvent. N_2 was purged with a flow rate of 60 mL/min throughout the addition of the metal solution. The resulting suspension was then transferred to a 125 mL Teflon-lined autoclave and was aged at 150 °C for 18 h at a static condition. At the end of aging, the precipitate was washed thoroughly with deionized water and kept in a state of wet gel for further utilization. The control-LDH sample was prepared in an aqueous solution using the same amounts and concentrations of the reagents, and the suspension after coprecipitation was aged at 60 °C for 24 h under continuous nitrogen purging and stirring.

6.2.2 Preparation of carbon nanosphere (CNS) template

CNS was prepared through carbonization of glucose under hydrothermal condition in an aqueous solution. Briefly, 4 g of D-glucose (Fisher, 99%) was dissolved in 42 mL of deionized water. The solution was then transferred to a 50 mL Teflon-lined autoclave and aged at 180 °C for 24 h. The resultant black slurry was centrifuged and washed with deionized water thoroughly and then dried at 60 °C overnight.

6.2.3 Direct assembly of LDH-NCs on the surface of CNS

The as-prepared wet gel of LDH-NCs (14 g of gel, equivalent to 1 g of dry basis LDH-NCs in 13 g of water) was dispersed in 25 mL of methanol by ultrasonication for 1 h to obtain a translucent and stable suspension. Then, this suspension was mixed with another mixture containing 250 mg of CNS powder in 25 mL of methanol. The resultant mixture was ultrasonicated at room temperature for 30 min. The LDH-NCs/CNS composite particles were afterwards collected by centrifugation at 3000 rpm and dried at 60 °C overnight. The assembly of the control-LDH sample on CNS was performed by the same procedure.

6.2.4 Generation of MgAl-oxide hollow nanospheres

The LDH-NCs/CNS and control-LDH/CNS composites were calcined in static air at 500 °C for 3 h with a slow heating rate of 1 °C/min to remove the CNS template. The obtained oxides were in a form of white powder, indicating that CNS cores were completely removed.

6.2.5 Formation of drug-intercalated LDH hollow nanospheres by reconstruction

The formation of LDH hollow nanospheres from their oxide precursor is based on the memory effect of LDH structure. Briefly, 4 mg of oxide powder was dispersed in 15 mL of ethylene glycol by ultrasonication for 30 min to obtain a stable suspension and was then

degassed by purging N₂ at 60 mL/min for another 30 min. The drug solution was prepared by dissolving 20 mg of ibuprofen sodium salt (Sigma, 99%) in 15 mL of ethylene glycol followed by degassing. Thereafter, the drug solution was added to the metal oxide suspension dropwise within 30 min. Once completed, 6 mL of degassed deionized water was added slowly to the mixture to aid the reconstruction process. The resulting suspension was then aged at 60 °C for 6 h under continuous N₂ purging and gentle magnetic stirring. At the end of aging, the product was collected by centrifugation, washed with ethanol and then dried at 60 °C overnight. In order to compare the drug release profile from LDHs of different morphologies, ibuprofen-intercalated Mg₂Al-LDH nanoplate-like sample (ibp-LDH nanoplates) was prepared by a co-precipitation method in an aqueous solution with aging at 70 °C for 3 d. The details about the preparation can be referred to experimental method in Chapter 4.

6.2.6 *In vitro* drug release study

The release of ibuprofen from ibp-LDH hollow nanospheres and ibp-LDH nanoplates was performed in an incubator at a constant temperature of 37 ± 0.5 °C by mixing 50 mg of dry powder samples in 150 mL of a phosphate buffer solution at pH 7.0 (Fluka, NaOH: 0.029 M; KH₂PO₄: 0.050 M) to simulate the body temperature and the blood stream condition. A continuous shaking at 150 rpm was applied. Ibuprofen sodium salt (Sigma, 99%) of approximately the same amount of that contained in ibp-LDH hollow nanospheres was tested under the same conditions for comparison. Aliquots of 2 mL were withdrawn from the suspensions at certain time intervals and then centrifuged to remove the particles. The clear supernatants were diluted to 2 times for measurement of ibuprofen concentrations using UV-vis spectroscopy at 264 nm. Each time after 2 mL of aliquots were withdrawn from the mixture, the same amount of the fresh buffer solution was replenished in order to keep the total volume of the mixture constant.

6.2.7 Materials characterization

Samples synthesized at various stages were characterized using dynamic light scattering, Zeta potential, TEM, SEM, XRD, FTIR, BET, TGA, ICP, CHN, and UV-vis techniques.

6.3 Results and Discussion

6.3.1 Characteristics of the precursor MgAl-LDH nanocrystals

Figure 6-2. (A) particle size distribution of LDH-NCs and (B) a translucent and stable suspension of LDH-NCs in methanol/water solvent.

The precursor MgAl-LDH nanocrystals (LDH-NCs) was synthesized using a similar method reported by Gursky, et al. by coprecipitating $\text{Mg}(\text{NO}_3)_2$ and $\text{Al}(\text{NO}_3)_3$ in pure methanol solvent.¹⁶⁷ Different from their ambient conditions during the aging, solvothermal treatment at 150 °C for 18 h was applied in our study, which results in smaller particle sizes. The particle size distribution was obtained by dynamic light scattering and it gives a narrow distribution with a mean value of 30 nm as shown in Figure 6-2A. Our particle sizes are also smaller than those (50-300 nm) of stable LDH nanoparticles prepared in aqueous media by Xu, et al. under hydrothermal conditions.²⁴⁷ This should be due to different solvation effects of methanol and water as the synthesis medium.²⁷⁴ The colloidal suspension of LDH-NCs in a methanol/water mixed solvent after thorough washing with deionized water was translucent

(Figure 6-2B) and remarkably stable for more than three months without undergoing aggregation. In contrast, the suspension of control-LDH sample was unstable and the particles quickly settled after stopping ultrasonication or stirring.

Figure 6-3. (A) TEM image LDH-NCs colloidal suspension, (B) and (C) SEM images of continuous thin film of LDH-NCs obtained by casting the colloidal suspension on to Silicon substrate.

TEM image shows that the sample consists of finely dispersed platelet-like nanocrystals (Figure 6-3A). In agreement with the results obtained by dynamic light scattering method, the particle sizes based on the TEM study are around 20-30 nm. Due to their narrow size distribution and good dispersity, LDH-NCs can form a stable colloidal suspension and continuous thin film when casted onto a Silicon substrate. The SEM images show the assembly of LDH nanocrystals to form a dense layer on the substrate (Figure 6-3B and 6-3C). As observed in these images, majority of LDH nanocrystals are assembled in the same orientation with their lateral surface sitting on top of one another, i.e., a face-to-face assembly. A similar phenomenon was also observed by Gursky, et al.¹⁶⁷

Figure 6-4. (A) TEM image control-LDH suspension, (B) and (C) SEM images of control-LDH particles obtained by casting the suspension on to Silicon substrate.

Different from LDH-NCs, control-LDHs exhibit severe aggregation. TEM image (Figure 6-4 A) illustrates the state of aggregation and a large extent of face-to-edge particle interactions. In addition, the particle size of control-LDHs is larger at around 60-100 nm. Based on the SEM images, the film formed by casting the suspension of control-LDH on the same substrate is porous and non-oriented. Large cavities between the particles can be observed (Figure 6-4B and 6-4C).

The powder XRD patterns of control-LDH and LDH-NCs are shown in Figure 6-5A and 6-5B, respectively. The results indicate the formation of the LDH structure with a rhombohedral ($3R$) symmetry.³⁰⁹ There is a small difference in the value of (003) basal spacing (d_{003}) between the two samples. The basal spacing d_{003} of control-LDH is 8.72 Å and that of LDH-NC is 8.95 Å. The estimated crystal size in c direction for control-LDH and LDH-NCs based on FWHM of their (003) basal planes is 12.3 and 9.8 nm, respectively.

Figure 6-5. XRD patterns of (A) control-LDHs and (B) LDH-NCs.

To identify the type of intercalated anions, the samples were subjected to FTIR analysis. Figure 6-6A displays the FTIR spectrum of control-LDH and it shows a sharp peak at 1384 cm^{-1} that is attributed to ν_3 vibration mode of NO_3^- with D_{3h} symmetry.³¹⁰ The metal-oxygen (M–O) bond is observed at 448 cm^{-1} , while the vibration and bending mode of the O–H group is indicated by a broad band at $3450\text{--}3550$ and 667 cm^{-1} , respectively. A peak at 1650 cm^{-1} is assigned to the bending mode of the adsorbed water molecules. Sample LDH-NC also exhibits these IR bands together with additional bands as shown in Figure 6-6B. The presence of methanol adsorbed in the interlayer gallery is indicated by absorption bands at 1054 and $2860\text{--}2980\text{ cm}^{-1}$, corresponding to the vibration of C–O and C–H, respectively.¹⁶⁷ Methoxide is first expected to be either intercalated in the interlayer space or possibly adsorbed on the surface of LDH-NCs because of the ionization of methanol in the highly

alkaline solution. During sample washing with deionized water, methoxide ions were hydrolyzed to become methanol and they could occupy the interlayer vacant site together with water molecules.

Since the (003) basal spacing obtained for both samples do not differ substantially, it is likely that nitrate is the main intercalated anion. Based on the elemental and thermal analysis results, the chemical formula determined for LDH-NCs is $\text{Mg}_{1.98}\text{Al}(\text{OH})_{5.96}(\text{NO}_3)_{0.71}(\text{CO}_3)_{0.15} \cdot 0.42\text{CH}_3\text{OH} \cdot 1.42\text{H}_2\text{O}$. The calculated compositions based on this formula agree well with the experimental values (Mg: 18.0%, Al: 10.1%, C: 2.5%, N: 3.7%, and H_2O : 10.2%).

Figure 6-6. FTIR spectra of (A) control-LDHs and (B) LDH-NCs.

6.3.2 Properties of LDH/CNS nanocomposites and metal oxide hollow nanospheres

Figure 6-7 shows the SEM image of CNS obtained by the hydrothermal reaction of glucose. The inset illustrates the abundance of hydroxyl groups present on the surface of CNS, which leads to a negative Zeta potential of -13.4 mV. Sun et al.³⁰⁷ and Titirici et al.³⁰⁵ showed that the surface of carbon spheres is hydrophilic and also has a rich distribution of C=O and C=C bonds. The presence of groups indicates that the carbon sphere formation is due to carbonatization and aromatization of glucose under hydrothermal condition. This carbonization step may arise from cross-linking induced by intermolecular dehydration of linear or branch-like oligosaccharides, or other macromolecules formed in prior step.³⁰⁷ The SEM picture shows a narrow size distribution of the obtained nanospheres at around 700-800 nm.

Figure 6-7. SEM image of carbon nanosphere (CNS) template. The inset illustrates the presence of abundant hydroxyl group on the surface.

The negatively charged surface of CNS can be directly coated with LDH nanocrystals via electrostatic interaction since the latter have a positive surface with a Zeta potential of +31.6 mV. Different from the conventional layer-by-layer technique, such a process is simple and can be performed without any additives or binding reagents. After coating with LDH

nanocrystals, Zeta potential of the resultant composite was measured +42.2 mV, indicating a good coverage of the nanocrystals on CNS.

SEM images of the dried composite sample (Figure 6-8A and 6-8B) indicate successful deposition of LDH-NCs on CNS by this simple procedure, as the surface of the nanospheres became rough after coating compared to the pristine smooth nanospheres. It is also observed that LDH nanocrystals successfully form dense layer on CNS surface and there are no free standing LDH particles observed although the amount of LDH-NCs used is excessive. For comparison, the control-LDH/CNS composite was also prepared using the same procedure. Based on the SEM images shown in Figure 6-8C and 6-8D, it is evident that the deposition is poor and many aggregates are formed separately, which leads to an incomplete coverage of CNS surface.

Figure 6-8. SEM images of (A), (B) LDH-NCs/CNS composites and (C), (D) control-LDH/CNS composites obtained by direct assembly of LDH particles on the surface of CNS in a single step without exfoliation.

Further characterizations of the composites by FTIR, XRD, and TGA methods confirm the presence of LDHs in the composites. As shown in Figure 6-9A, the presence of LDHs is indicated by the small peak at 1383 cm^{-1} which is attributed to the vibration mode of the intercalated nitrate ion. Other major bands are attributed to CNS. The absorption at 1700 and 1620 cm^{-1} corresponds to the vibration mode of $\text{C}=\text{O}$ and $\text{C}=\text{C}$ bonds, respectively.³⁰⁷ In addition, the XRD pattern (Figure 6-10A) mainly gives the diffraction of the amorphous phase of CNS indicated by a broad peak around 20° . The diffraction signal from the LDH phase is too weak to be observed clearly. The content of the adsorbed LDH/NCs on CNS surface was roughly estimated based on TGA result. Figure 6-11A and 6-11B shows the thermal degradation of pristine LDH-NCs and LDH-NC/CNS composite, respectively. The weight loss from room temperature up to 200°C is due to desorption of the adsorbed and intercalated water molecules. Sample LDH-NCs displays two degradation steps at $300\text{--}400^\circ\text{C}$ and 500°C . The former is due to dehydroxylation of LDH layers and decomposition of the adsorbed methoxide ion that count for 32.06% of weight loss and the latter is attributed to the decomposition of nitrate anion that corresponds to 7.53% weight loss. The final percentage residue is 49.26%, which consists of MgAl mixed oxide. On the other hand, the LDH-NCs/CNS nanocomposite shows a continuous weight loss starting from 300°C to 550°C mainly due to gradual decomposition of CNS. The residual weight of 4.67% accounts for the final MgAl mixed oxide. Based on these data, it can be calculated that the weight percentage of LDH nanocrystals adsorbed on the CNS surface is about 9.5%. The obtained low yield suggested that most of the LDH nanocrystals remain in the colloidal suspension, as the initial LDH : CNS weight ratio was 4:1.

Figure 6-9. FTIR spectra of (A) LDH-NCs/CNS composites and (B) MgAl-oxide hollow spheres obtained after calcination of LDH-NCs/CNS composites.

Figure 6-10. XRD patterns of (A) LDH-NCs/CNS composites and (B) MgAl-oxide hollow nanospheres.

Figure 6-11. TGA results of (A) LDH-NCs and (B) LDH-NCs/CNS composites with the residue weight percentages indicated at the end of each curve.

The obtained composite nanospheres were then calcined at 500 °C to remove the CNS core. The heating rate was set 1°C, which is slow enough to prevent the rupture of the spheres. The SEM and TEM images of the resulting oxide after calcination of LDH-NCs/CNS (Figure 6-12A and 6-12B, respectively) exhibit the morphology of hollow nanospheres with their diameters reduced to around 300-400 nm. The shell thickness of the oxide hollow nanospheres is ca. 40 nm. The thickness of the shell can be controlled by varying the amount of LDH nanocrystals in the suspension. The formation of mixed oxide was confirmed by FTIR and XRD results as presented on Figures 6-9B and 6-10B, respectively. The FTIR bands at 1414 and 1494 cm^{-1} are attributed to the vibration of carbonate adsorbed reversibly on the oxide surface.³¹¹ The band at 441 cm^{-1} is assigned to vibration of metal-oxygen (M-O) bond. The XRD pattern shows (200) and (220) peaks, which can be assigned to cubic phased MgO (PDF card no. 45-0946), and the (111) peak that is due to the trace cubic spinel oxide²³⁴ present in MgAl mixed oxide hollow nanospheres. The low intensities exhibited by the XRD pattern indicate the formation of amorphous material. Besides, it could also be due to small crystallites size of the obtained mixed oxide.

Figure 6-12. SEM (A) and TEM (B) images of MgAl-oxide product formed after calcination of LDH-NCs/CNS, SEM (C) and TEM (D) images of MgAl-oxide product formed after calcination of Control-LDHs/CNS composites.

It is very interesting that the spherical shape with good structural integrity is well preserved even with a large volume contraction (~50% of reduction in diameter) from the composite precursor to the oxide. This should be attributed to the densely packed pristine LDH-NCs on CNS from the stable colloidal suspension. During the heat treatment, the nanocrystals were transformed to oxides, which cross-linked to form the continuous and porous shell of the hollow nanospheres. In contrast, the metal oxide prepared under the same conditions but using the control-LDH suspension, which is non-stable, gave broken hollow spheres with fragmented shell walls (Figure 6-12C and 6-12D).

Figure 6-13. Adsorption/desorption isotherm (A) and the pore size distribution (B) of the resulting hollow MgAl-oxide.

The nitrogen adsorption-desorption isotherm of the obtained hollow oxide is presented in Figure 6-13A together with the pore size distribution (Figure 6-13B). It is shown to exhibit type II sorption based on Brunauer, Demming, Demming, and Teller (BDDT) classification, which typically does not present a plateau at high P/P_0 , and it shows hysteresis loop type H3.²²⁰ Similar result is also obtained elsewhere, where coprecipitation method was used to prepare LDHs.^{119, 312-313} Although the typical type II isotherm does not usually possess any hysteresis, the presence of type H3 desorption loop indicates the presence of non-rigid aggregates of plate-like particle that give rise to slit-shaped pores,²²⁰ which is the characteristic of clay minerals, such as LDHs.¹¹⁹ Nevertheless, the narrow hysteresis loop may evidence a narrow range of pore dimensions.²³⁰ The BJH desorption pore size distribution indicates the presence of mesopores, with most of them measured 9.53 nm and a small fractions or pores in the range of 2-4 nm. The surface area is determined by multipoint BET method, giving the value of 151.7 m²/g, which is comparable to those calcined products reported by others.^{230, 313-314}

6.3.3 Formation of drug-LDH hollow nanospheres by reconstruction

Figure 6-14. SEM (A) and TEM (B) images of ibuprofen-intercalated LDH hollow nanospheres after reconstruction. Inset shows the shell of a hollow nanosphere.

The reconstruction of the resultant oxide product to LDHs in the presence of drug anions was performed in ethylene glycol with the controlled amount of water. A controlled recrystallization rate is regarded important in order to preserve the hollow nanosphere structure. As LDHs naturally grow into 2-D plate-like anisotropic particles, quick recrystallization may lead to the fast formation of these plates, which could disrupt the spherical structure. It has been identified that the temperature and amount of water are the two critical parameters for successful reconstruction of drug intercalated LDHs while maintaining their hollow nanosphere structure. Figure 6-14 presents SEM and TEM images of ibuprofen (a model drug)-intercalated LDH hollow nanospheres obtained by reconstruction. The shell thickness increased to about 80 nm due to the formation LDH structure with expanded interlayer spacing by the drug anions.

Figure 6-15. XRD pattern (A), FTIR spectrum (B) of Ibp-LDH hollow nanospheres.

The XRD and FTIR results (Figure 6-15) confirm the formation of ibuprofen-intercalated LDH (ibp-LDH). The d -spacing of ibp-LDH hollow nanospheres calculated from the (003) peak is 22.0 Å which is consistent with that previously reported.²²¹ The FTIR spectrum shows the presence of ibuprofen anion by the peaks at 1559 and 1398 cm⁻¹ due to antisymmetric and symmetric stretching of $RCOO^-$ group, respectively. The alkyl stretching of ibuprofen is observed at 2873 and 2956 cm⁻¹. The content of the intercalated ibuprofen in the hollow nanospheres was found at 36.8% by UV-vis measurement. Based on the combined ICP, CHN and thermal analysis results, the estimated formula of ibp-LDH hollow nanospheres is $Mg_{1.99}Al(OH)_{5.98}(Ibp)_{0.65}(CO_3)_{0.17} \cdot 2.40H_2O$. The presence of carbonate could be due to contamination from atmospheric CO₂.

The adsorption-desorption isotherm and the pore size distribution of the regenerated hollow Ibp-LDH is presented in Figure 6-16A and 6-16B, respectively. Similar to that of hollow oxide, the regenerated Ibp-LDH also exhibits type II with a narrow hysteresis loop of type H3.²²⁰ BJH method desorption pore diameter shows a unimodal and narrow distribution of mesopores peaked at 4.2 nm. The surface area is determined by multipoint BET method gives the value of 53.9 m²/g, which is about one-third less than that of the hollow oxide, but is higher than the conventional Ibp-LDH nanoplates (14.7 m²/g) synthesized by coprecipitation.

Such result is expected as the individual oxide particles reconstruct to form larger sized plate-like LDHs.

Figure 6-16. Adsorption/desorption isotherm (A) and the pore size distribution (B) of the resulting ibp-LDH hollowspheres.

Our results indicate that the oxide hollow nanospheres are sufficiently robust to withstand ultrasonication, gas bubbling, and magnetic stirring during the reconstruction process in the solution phase. Such characteristics provide good opportunities of forming LDH hollow nanospheres intercalated with functional anions and further entrapping other molecules into the hollow interior to make multifunctional nanospheres.

Figure 6-17 shows the *in vitro* release profile of ibuprofen from ibp-LDH hollow nanospheres, in comparison with those from ibp-LDH nanoplates and the simple ibuprofen salt (inset). It is found that there is no substantial difference in the release profiles for the two types of LDH samples. After 5 min, a release percentage of 50% was detected, and 90% after around 40 min for both samples, while the ibuprofen salt was dissolved instantaneously. However, there still exist several advantages for the hollow nanosphere LDH sample, such as i) a lower density and less aggregation compared to the nanoplates, which leads to a better dispersion in the liquid phase (as shown in Figure 6-18) for potential applications in controlled drug release with an intravenous injection mode; ii) a higher surface area (53.9 m^2/g compared to 14.7 m^2/g for ibp-LDH nanoplates) allows more effective surface

modification by functional species (e.g., polymers, silica, etc.); and iii) the interior space of the hollow nanospheres can be used for encapsulation of other molecules or nanoparticles (e.g., dyes, magnetic nanoparticles, fluorescent materials, etc.) to make multifunctional nanocomposites.

Figure 6-17. Ibuprofen release profile from LDH hollow nanospheres with inset showing the release profile of ibuprofen from conventional plate-like LDH.

Figure 6-18. Photos taken for ibuprofen-intercalated LDH suspensions in deionized water: comparison between Ibp-LDH nanoplates (A) and Ibp-LDH hollow nanospheres (B).

6.4 Conclusion

The work presented in this chapter demonstrates the direct assembly of preformed anisotropic nanocrystals onto a spherical template in a single step for the generation of hollow nanospheres. A key requirement identified to achieve the high quality hollow nanospheres is the use a stable colloidal suspension of finely dispersed LDH nanocrystals to prevent self-aggregation of LDHs particles. It is anticipated that this approach will open a new avenue to fabricate core/shell nanocomposites and hollow structures by avoiding the tedious procedures involving the conventional exfoliation/LBL-stacking process.”

CHAPTER 7 Tb(III)-DOPED LAYERED DOUBLE HYDROXIDES AND THEIR PHOTOLUMINESCENCE PROPERTIES

(This chapter is reproduced with permission from “Gunawan, P. and Xu, R., *Lanthanide-doped Layered Double Hydroxides Intercalated with Sensitizing Anions: Efficient Energy Transfer between Host and Guest Layer*. Journal of Physical Chemistry C, 113, 17206-17214, 2009.” Copyright 2009, The American Chemical Society)³¹⁵

7.1 Introduction

Synthesis of nanomaterials with several functionalities has been gaining increasing attention recently since they are able to serve different purposes simultaneously. In biomedical applications, the use of multifunctional materials as pharmaceutical carriers is able to offer a new platform for both diagnostic and treatment of diseases as development of such smart nanocarriers will provide a desired combination of useful properties.^{56, 316-317} By having combination of several functionalities together in one system, it may significantly improve the effectiveness of drug performance and reduce their negative side effects.

As potential hosts for drug molecules, LDHs can be further functionalized to improve their performance. Some attempts have been made to achieve such a target by combining iron oxide nanoparticles with LDHs to form a magnetic-LDH hybrid.³¹⁸⁻³¹⁹ Photoluminescent agents are also incorporated in the interlayer galleries of LDHs for imaging applications. Successful immobilization of a rhodamine B into LDH pre-intercalated with dodecylbenzene sulfonate (DBS) was reported by Yan et al.³²⁰ In another report, Mohanambe et al. utilized SDS- and β -cyclodextrin-functionalized LDHs to incorporate pyrene and naphthalene, respectively.³²¹⁻³²² Besides organic compounds, lanthanide ions (Ln^{3+}) have also been widely used as imaging species, and the intercalation of their complexes into LDH galleries have

been reported by a number of research groups. Xu et al. intercalated Gd(III)-diethyl triamine pentaacetate (DTPA) complex into the interlayer of MgAl-LDH to improve the performance of Gd(III) in magnetic resonance imaging (MRI).³²³ Chang et al intercalated dipicolinate complex of Eu^{3+} and Ce^{3+} .¹⁸² Gago et al. employed different ways to intercalate Eu and Gd into ZnAl-LDHs pillared with 2,2'-bipyridine-5,5'-dicarboxylate (BDC) as a porous matrix..³²⁴

The use of rare earth elements for biological applications, especially Yb^{3+} , Eu^{3+} , Er^{3+} , and Tb^{3+} as doping materials to impart photoluminescence properties has been widely investigated recently.^{317, 325-328} They provide good alternatives besides organic phosphors and quantum dots due to several reasons: i) their emissions are sharp and much more photostable than those of organic fluorophores, ii) they exhibit long luminescence lifetime (μs -ms) while that of dye molecules is in the order of ns,³²⁹ iii) they are of multiphotons so as to allow up-conversion excitation,³³⁰⁻³³¹ and iv) they possess low toxicity over semiconductors quantum dots nanomaterials.

However, in previous studies, the anionic lanthanide complexes are incorporated in the interlayer as charge compensating species, and therefore, other functional molecules (e.g. drug anions) can no longer be intercalated. Such a drawback will limit the utilization of these luminescent LDHs in biomedical applications. Therefore, another strategy was developed to introduce rare earth cations in the crystal lattice of brucite-like layers, while incorporating other active molecules, such as drug compounds, in the interlayer. By doing so, a novel luminescent LDH containing drug molecules is attained, thus giving rise to the capability of LDH in therapeutic field as both drug carrier and imaging agent. To the best of our knowledge, there are only a few publications reporting the inclusion of rare earth ions in the brucite-like layers. Fernandez et al. reported the synthesis of Mg-Al-LDH containing Yttrium (Y^{3+}).¹⁸⁵ Das et al. synthesized Mg-Al LDHs containing Cerium (Ce^{3+}).¹⁸⁷ They studied the effect of the presence of rare earth ions on the crystallinity of the resulting LDHs and

their thermal stability. An in-depth study on Eu-doped MgAl-LDHs using TRLFS and EXAFS measurement was reported by Stumpf et al.³³² The study revealed that Eu^{3+} was included into the bulk structure of material and it induced distortion in crystal lattice of the brucite-like layer. Recently, Gao et al. prepared a novel red-emitting material, $\text{Ca}_3\text{Al}_2\text{O}_6:\text{Eu}^{3+}$ by calcination of Eu^{3+} -doped LDH as precursor.¹⁸⁸ It was found that its luminescence is dependent on the calcination temperature.

In the present study, terbium(III) was introduced into the LDH layers as it is one of the most emissive lanthanide ions.³³³ A systematic study on the effect of Tb^{3+} content on the LDH characteristics and the luminescence properties is presented in this chapter. The effects of the intercalated species as well as different host materials on Tb^{3+} emission properties and the mechanism of energy transfer are discussed in detail. In addition, the stability of LDH layers in confining Tb^{3+} cations was also investigated to observe its feasibility as drug carriers and photoluminescent agents.

7.2 Experimental Method

7.2.1 Synthesis of drug loaded MgAlTb-LDHs

The synthesis of drug loaded MgAlTb-LDH was carried out in a simple one-pot procedure using the coprecipitation method. All chemicals used for the synthesis are of analytical grade and were used without further purification. Briefly, 20 mL of aqueous solution mixture of 0.45 M magnesium nitrate hexahydrate ($\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 99%, BDH), aluminium nitrate nonahydrate ($\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, 99%, ACROS Organics), and terbium (III) chloride hexahydrate ($\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$, 99.9%, Sigma-Aldrich) was added drop by drop at room temperature into 80 mL of vigorously stirred alkaline solution (NaOH , >99%, pellet, Merck) mixed with biphenylacetic acid (BPA, 98%, ACROS). Both solutions were prepared in deionized water. The molar ratio of divalent cation (Mg^{2+}) to trivalent cations (Al^{3+} and Tb^{3+}) was maintained at 2 while varying the molar ratio of $\text{Tb}^{3+}:\text{Al}^{3+}$ at 0.0, 0.05, 0.2 and 1.0.

Accordingly, the samples synthesized were denoted as NoTb-BPA, Al₂₀Tb-BPA, Al₁₅Tb-BPA, and Al₁₁Tb-BPA, respectively. The molar amount of NaOH was twice as that of the mixed salts and the drug compound was 3-fold in excess of trivalent cations. Beside BPA, other drug compounds with different structures were used in the synthesis, such as valproic acid, 5-fluorouracil and folic acid. The salt feeding rate was kept around 0.67 mL/min and the stirring rate was 300 rpm. Before the addition of salt solution, the solution of NaOH and drug mixture was bubbled with nitrogen at 60 mL/min for 30 min to remove carbon dioxide from the synthesis chamber and the purging was continued during precipitation and aging period. After addition of salt solution was complete, the suspension was aged in the same reaction flask for 48 hr at 70 °C. At the end of aging, the suspension was centrifuged and the precipitate was washed thoroughly with deionized water followed by drying in the oven at 70 °C overnight.

7.2.2 Synthesis of nitrate-intercalated MgAlTb-LDHs as control

The procedure for synthesis of nitrate-containing MgAlTb-LDH was similar to those of drug-loaded LDHs as described in the preceding subsection in the absence of drug compounds. The Tb³⁺:Al³⁺ molar ratio was only varied at 0.0, 0.05 and 0.2. The resulting products were denoted as NoTb-NC, Al₂₀Tb-NC, and Al₁₅Tb-NC, respectively.

7.2.3 Synthesis of terbium(III) 4-biphenylacetate salt

The salt of terbium(III) 4-biphenylacetate (Tb-BPA) was synthesized via a simple precipitation method for comparison purpose. Briefly, 20 mL of 0.025 M TbCl₃·6H₂O was added drop wise into 80 mL of vigorously stirred aqueous solution containing NaOH and 4-BPA each with a concentration of 0.0188 M. The resulting precipitate was aged at 70 °C for 48 h. The product was obtained by centrifugation and washed thoroughly with deionized water followed by drying in oven at 70 °C overnight.

7.2.4 Ion exchange of zeolite Na-Y with Tb(III) and adsorption of BPA

The experimental procedure can be referred to that reported by Wada et al.³³⁴ with some modification. Briefly, the ion exchange of Na⁺ with Tb³⁺ was carried out by stirring 200 mg of commercial zeolite Na-Y in 25 mL of an aqueous solution of TbCl₃·6H₂O (0.115 M) at 80 °C for 16 h. The precipitate was collected by centrifugation and washed with deionized water followed by drying.

The Tb³⁺-exchanged sample was then degassed overnight to remove the entrapped water molecules. BPA adsorption was then carried out by mixing the degassed powder with 0.3 g of BPA in 25 mL of ethanol. The suspension was stirred at room temperature for 20 h before centrifugation and drying.

7.2.5 Photoluminescence study

Shimadzu RF-5301 PC spectrofluorophotometer with 150W Xenon lamp was used to observe luminescence emission. Around 2 mg of the dry sample was ground into fine powder and dispersed in 10 mL of methanol. Ultrasonication was applied for about 1.5 h to make a homogeneous suspension. The emission spectra were collected at different wavelengths, corresponding to the maximum wavelength obtained from the excitation spectra. The excitation and emission slits used were 5 nm and 1.5 nm, respectively.

The luminescence decay curves were collected using Fluorolog Jobin-Yvon-SPEX equipped with a flash lamp source with both excitation and emission slits of 8 nm. Excitation wavelengths were set at 233 nm and 275 nm while emission wavelength was set at 545 nm.

Quantum yield of samples was determined using the following calculation:²¹⁴

$$Q_S = Q_R \frac{I_S}{I_R} \frac{OD_R}{OD_S} \frac{n_S^2}{n_R^2} \quad (\text{eq. 7-1})$$

where the subscripts *S* and *R* denote the sample under study and the reference standard, respectively; *Q* is the quantum yield; *I* is the integrated intensity; *OD* is optical density/absorbance, and *n* is the refractive index. A solution of cresyl violet in methanol with

a dilute concentration of 10^{-7} M was used as reference which has a quantum yield of 0.54.³³⁵ The primary and secondary filter effects of the fluorescence intensity were approximately corrected by equation 7-2:²¹⁴

$$I_{corr} = I_{obs} 10^{[OD(\lambda_{exc}) + OD(\lambda_{em})]/2} \quad (\text{eq. 7-2})$$

where $OD(\lambda_{exc})$ and $OD(\lambda_{em})$ are the optical densities of the suspension at the excitation and the emission wavelength, respectively.

7.2.6 Leaching study of cations from LDH layers

During the leaching study, 25 mg of dry sample of MgAlTb-LDH ($\text{Tb}^{3+}/\text{Al}^{3+} = 0.2$) containing BPA was suspended in 25 mL of deionized water using ultrasonication for 2 h. The suspension was then incubated at room temperature with continuous shaking at 180 rpm for 5 d. The final suspension was then centrifuged at 12000 rpm for 5 min. The supernatant and the solid particles were collected for determination of the amount of cations leached out from the LDH layers. The above experiment was also conducted for sample MgAl-BPA-LDH after physically adsorbed with Tb^{3+} ions for comparison.

7.2.7 Materials characterizations

The samples were subjected to various characterization methods, such as XRD, FTIR, TEM, EDS, ICP, TGA, CHN elemental analysis, and UV-vis spectrophotometer, as described in Chapter 3.

7.3 Results and Discussion

7.3.1 Physicochemical properties of Tb(III)-doped LDHs

Table 7-1. Experimental conditions of the prepared samples and their crystallographic parameters.

Sample name	Anion	Precursor solution		Crystallographic parameters			
		Mg : (Al+Tb) molar ratio	Tb : Al molar ratio	d_{003} (Å)	a (Å)	$\text{FWHM}_{(003)}$	Dp_{003} (Å)
NoTb-NC	nitrate	2.00	0.00	8.62	3.035	0.457	174.53
Al20Tb-NC	nitrate	2.00	0.05	8.83	3.036	1.188	67.13
Al5Tb-NC	nitrate	2.00	0.20	8.69	3.036	1.198	66.57
NoTb-BPA	4-biphenylacetate	2.00	0.00	20.63	3.032	0.520	152.88
Al20Tb-BPA	4-biphenylacetate	2.00	0.05	21.63	3.030	0.948	83.86
Al5Tb-BPA	4-biphenylacetate	2.00	0.20	22.64	3.034	0.988	80.45
Al1Tb-BPA	4-biphenylacetate	2.00	1.00	25.89	3.078	1.012	78.54

Figure 7-1. XRD pattern (left) and FTIR spectra (right) of Al5Tb-NC (A), Al20Tb-NC (B) and NoTb-NC (C).

Figure 7-2. XRD pattern (left) and FTIR spectra (right) of Al1Tb-BPA (A), Al5Tb-BPA (B), Al20Tb-BPA (C), and NoTb-BPA (D).

Table 7-1 summarizes the experimental parameters and the crystallographic information of the LDH samples prepared. The initial molar ratio of $\text{Tb}^{3+}:\text{Al}^{3+}$ in the precursor solutions was varied while keeping $\text{M}^{2+}:\text{M}^{3+}$ fixed at 2.0. XRD patterns and FTIR spectra of samples containing nitrate are shown in Figure 7-1. Diffraction patterns of all samples can be indexed according to $3R$ rhombohedral symmetry. Compared to sample NoTb-NC, which exhibits a sharp and well-resolved XRD pattern, the introduction of Tb^{3+} in the brucite-like layers results in a less ordered stacking of the brucite-like layers and a lower crystallinity of the resultant products. As observed in Figures 7-1A and 7-1B, the basal peaks were broadened and the resolution of (110) peak became poorer with an increasing content of Tb^{3+} . This should be due to the deformation of the brucite-like layers caused by incorporation of large Tb^{3+} cations into the crystal lattice. In addition, the formation of $\text{Tb}(\text{OH})_3$ was observed (marked with *) when the $\text{Tb} : \text{Al}$ ratio is 0.2. According to PDF no. 19-1325, the formed hydroxide is hexagonal $\text{Tb}(\text{OH})_3$ with $P6_3/m$ symmetry. Since the octahedral ionic radius of Tb^{3+} (0.923 Å) is much larger than that of Al^{3+} (0.535 Å),³³⁶ there seems a certain maximum amount of Tb^{3+} that can be accommodated in the octahedral lattice of LDH layers. Further increase of the Tb^{3+} molar percentage leads to the formation of a segregated $\text{Tb}(\text{OH})_3$ phase. The basal spacings (d_{003}) of samples NoTb-NC, Al20Tb-NC, and Al5Tb-NC obtained from their respective (003) peak are 8.62 Å, 8.83 Å, and 8.69 Å, respectively. They are in

good agreement with that obtained by Xu et al. for nitrate-intercalated LDH with Mg^{2+} : Al^{3+} of 2.0.³³⁷

The effect of Tb^{3+} incorporation in the hydroxide layers is also observed on FTIR spectra. The absorption band at 448 cm^{-1} , which is attributed to metal-oxygen (M–O) vibration bond in the octahedral lattice, becomes weaker with the inclusion of Tb^{3+} . Since the intensity of an infrared absorption band is dependent on the magnitude of the change of dipole moment,²³³ replacing the constituent atom may change the dipole moment since it affects the charge and the bond distance between the atoms. As Al^{3+} cations in the LDH layers are partially replaced by Tb^{3+} , which is of larger size and possesses smaller polarizing ability, dipole moment of M–O (metal-oxygen) bonds may change and thus affects the absorption of the infrared, either reducing the absorbance intensity or further shifting the absorption band. Reports by Xu et al.^{234, 310} indicate that the intensity of M–O vibration band is first weakened and then shifted to higher wavenumber when the amount of Al is increasing. The presence of the interlayer nitrate anion is indicated by a sharp peak at 1384 cm^{-1} . Other bands at 1630 cm^{-1} and around 3500 cm^{-1} are assigned to δ -mode of the adsorbed water molecules and the vibration of the hydroxyl groups of brucite-like layers, respectively.

A successful intercalation of BPA is demonstrated by XRD and FTIR as displayed in Figure 7-2. The molar ratio of Tb^{3+} : Al^{3+} was varied at 0, 0.05, 0.2, and 1.0. The XRD patterns indicate the formation of a single crystalline LDH phase as the presence of $\text{Tb}(\text{OH})_3$ is not observed even when a large amount of Tb^{3+} was introduced (sample Al1Tb-BPA). When more Tb^{3+} cations are being incorporated, a gradually increasing basal spacing from $20.63\text{ \AA}$ (NoTb-BPA) to $25.89\text{ \AA}$ (Al1Tb-BPA) was resulted. The increase in basal spacing could be related to a smaller Coulombic interaction between the positively charged brucite-like layers and negatively charged interlayer anion due to a smaller polarizing ability of Tb^{3+} . A similar phenomenon was also observed by Das et al. when cerium cations were incorporated into the LDH lattice.¹⁸⁷ The grain size in c direction (D_{p003}) calculated using

Scherrer formula²¹⁰ is found decreasing with an increasing Tb^{3+} content. The estimated number of brucite-like layers is 7.4, 3.9, 3.5, and 3.03 for samples NoTb-BPA, Al₂₀Tb-BPA, Al₁₅Tb-BPA, and Al₁₁Tb-BPA, respectively. In addition, the arrangement of atoms in brucite-like layers is also affected based on the observation of the disappearing (110) peak as more Tb^{3+} cations were incorporated.

FTIR spectra in Figure 7-2 show that the vibration of M–O band shifts from a higher frequency at 448 cm^{-1} , when less Tb^{3+} was introduced, to lower one at 402 cm^{-1} when $\text{Tb}^{3+}/\text{Al}^{3+}$ was 1.0. The larger size of Tb^{3+} compared to that of Al^{3+} leads to a weaker bond, and subsequently the vibration band shifts to a lower frequency. Similar to those of nitrate-intercalated LDHs, the intensity of the absorption band at 448 cm^{-1} also decreases with an increasing amount of Tb^{3+} . The presence of BPA is evidenced by antisymmetric and symmetric stretching vibration mode of carboxyl group at 1559 and 1395 cm^{-1} , respectively. However, the spectrum of sample Al₁₁Tb-BPA shows that nitrate anions co-exist in the interlayer as indicated by the absorption peak at 1384 cm^{-1} .

The elemental mapping of Mg, Al, and Tb in selected samples was obtained by electron energy loss spectroscopic (EELS) technique and is presented in Figure 7-3. The concentration of each element, which is indicated by the relative brightness and the intensity of the color, corresponds to its composition in the sample. In general, all three elements exhibit a homogeneous distribution across the BPA intercalated LDH samples. It can be observed that upon varying the ratio of $\text{Al}^{3+}:\text{Tb}^{3+}$, the concentration of Al^{3+} and Tb^{3+} in the samples varies accordingly. The results therefore suggest a successful incorporation of Tb^{3+} cations in the LDH crystal lattice. On the other hand, one may note that the incorporation of Tb^{3+} in nitrate-intercalated LDH (Al₁₅Tb-NC, Figure 7-3A) is not as successful as that in BPA-intercalated LDH (Al₁₅Tb-BPA, Figure 7-3C) even though the same amount of Tb^{3+} was used during the sample preparation. The EELS image of the former shows a weaker signal of Tb^{3+} . In addition, the signal comes not only from the particles in the TEM image but also

from areas outside the boundary of the particles where some amorphous $\text{Tb}(\text{OH})_3$ phase could be present.

Figure 7-3. Electron energy loss spectroscopy (EELS) images of Mg, Al, and Tb for samples A15Tb-NC (A), A120Tb-BPA (B), A15Tb-BPA (C), and A11Tb-BPA (D).

The immobilization of lanthanide ions, in general, either through complexation with ligands or incorporation in a host material is important because they are highly labile, which may be detrimental to some *in vivo* application.³³⁸ Confinement of Tb^{3+} cations in LDH lattice may improve their stability and biocompatibility. The leaching test was performed by

dispersing Tb-containing LDH powder (Al₅Tb-BPA) in deionized water followed by incubation at room temperature for 5 days. As a control, MgAl-BPA-LDH that was physically adsorbed with Tb³⁺ ions (MgAl-BPA+Tb) was prepared and it was incubated at the same condition as that of Al₅Tb-BPA. The concentrations of metal cations leached from LDHs to the supernatants are shown in Table 7-2. It can be seen that the physically adsorbed Tb³⁺ cations easily leached out and a concentration of around 12.3 mg/L was reached at the end of incubation. On the other hand, the confinement of Tb³⁺ in LDH layers significantly improved its stability and a very low concentration of 0.07 mg/L was measured for Tb³⁺. With an average blood volume per body weight for normal adult as 85 ml/kg³³⁹, the amount of leached Tb³⁺ from LDH layers per body weight is estimated as low as 6 µg/kg (body weight). A study on the mortality by Hirano et al. shows that rare earth elements are not highly toxic. The LD₅₀ value for intravenous injected rare earth is ranging from 10 to 100 mg/kg³⁴⁰, depends on the type of rare earth elements and their compounds. However, the calculated amount of leached Tb³⁺ is still much less than the mentioned range, therefore, the incorporation of Tb³⁺ into LDH help increase its biocompatibility under neutral environment, such as in the physiological system. Besides Tb³⁺, a small amount of Mg²⁺ was leached out from both samples, since Mg²⁺ has the highest solubility compared to the other two cations.¹¹⁷

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Table 7-2. Composition of supernatant after leaching test study.

Sample name	Mg ²⁺ (mg/L)	Al ³⁺ (mg/L)	Tb ³⁺ (mg/L)
Al ₅ Tb-BPA	5.674	0.000	0.070
MgAl-BPA+Tb	2.056	0.054	12.296

The compositions and the chemical formulae of the samples are presented in Table 7.3. It is noted that the molar ratio of Tb³⁺: Al³⁺ is always larger than that in the precursor solution, while that of M²⁺: M³⁺ is close to two in all the samples. Fernandez et al.¹⁸⁵ and Das et al.¹⁸⁷

incorporated MgAl-LDHs with yttrium and cerium, respectively, and both found RE^{3+} : Al^{3+} smaller than that in the precursor solutions. In a recent work by Musumeci et al., the Tb^{3+} : Al^{3+} ratio was found similar to that in the precursor solution.³⁴¹ The discrepancies could be due to different synthesis conditions. As the aging time used in our experiment was long enough (48 h), it may help facilitate the inclusion of the relatively large Tb^{3+} cations into brucite-like layers.

The study on the particle morphology with and without incorporation of Tb^{3+} was performed with TEM analysis (Figure 7-4). The Tb^{3+} -free MgAl-LDHs containing nitrate (Figure 7-4A) and BPA anion (Figure 7-4C) exhibit distinct 2-D plate-like morphology of 50-70 nm and 40-50 nm in size, respectively. After incorporation of Tb^{3+} , it is observed that the nanoplates become more irregularly shaped and agglomerated to form clusters. Based on the XRD analysis results shown earlier (Table 7-1), the number of brucite-like layers in the nanoplates decreased with an increasing Tb^{3+} content. Since the surface effect becomes dominant for thin nanocrystallites, the LDH nanoplatelets become more flexible with higher surface energy, which promotes their agglomeration to form large aggregates.

Figure 7-4. TEM images of NoTb-NC (A), Al5Tb-NC (B), NoTb-BPA (C), Al20Tb-BPA (D), Al5Tb-BPA (E), and Al1Tb-BPA (F).

Table 7-3. Elemental analysis and chemical compositions of samples listed in Table 7-1.

Sample name	ICP					CHN analysis			UV-Vis	TGA	Chemical Formulae
	Mg : (Al+Tb) molar ratio	Tb : Al molar ratio	%Mg	%Al	%Tb	%C	%H	%N	%BPA	%H ₂ O	
NoTb-NC	1.97	0.00	19.91	11.13	0.00	0.43	3.94	3.67	N.A. ^a	10.22	Mg _{0.67} Al _{0.34} (OH) ₂ (NO ₃) _{0.23} (CO ₃) _{0.05} · 0.48H ₂ O
Al20Tb-NC	2.04	0.06	19.12	9.82	3.33	0.88	3.68	2.94	N.A. ^a	12.05	Mg _{0.67} Al _{0.31} Tb _{0.018} (OH) ₂ (NO ₃) _{0.184} (CO ₃) _{0.071} · 0.59H ₂ O
Al5Tb-NC	2.05	0.21	16.94	7.60	9.25	1.06	3.54	2.68	N.A. ^a	12.79	Mg _{0.67} Al _{0.27} Tb _{0.056} (OH) ₂ (NO ₃) _{0.18} (CO ₃) _{0.074} · 0.67H ₂ O
NoTb-BPA	1.95	0.00	9.77	5.57	0.00	37.84	5.34	0.00	46.40	13.26	Mg _{0.66} Al _{0.33} (OH) ₂ (BPA) _{0.32} (CO ₃) _{0.007} · 1.09H ₂ O
Al20Tb-BPA	2.33	0.06	12.49	5.60	2.02	35.89	5.10	0.10	44.53	6.85	Mg _{0.70} Al _{0.28} Tb _{0.017} (OH) ₂ (BPA) _{0.28} (NO ₃) _{0.009} (CO ₃) _{0.007} · 0.49H ₂ O
Al5Tb-BPA	2.17	0.27	10.84	4.50	6.89	37.04	4.82	0.00	48.23	9.73	Mg _{0.69} Al _{0.25} Tb _{0.07} (OH) ₂ (BPA) _{0.34} · 0.84H ₂ O
Al1Tb-BPA	2.14	1.13	10.91	2.83	19.54	28.18	3.95	0.47	34.54	7.77	Mg _{0.68} Al _{0.15} Tb _{0.17} (OH) ₂ (BPA) _{0.24} (NO ₃) _{0.05} (CO ₃) _{0.013} · 0.64H ₂ O

^a These samples does not contain BPA anion.

7.3.2 Photoluminescence properties of Tb(III)-doped LDHs intercalated with BPA anion

Figure 7-5. Excitation spectra ($\lambda_{\text{EM}} = 545$ nm) of samples NoTb-BPA (A), Al₂₀Tb-BPA (B), Al₅Tb-BPA (C), Al₁Tb-BPA (D), Tb-BPA (E), NoTb-NC (F), Al₅Tb-NC (G), ZY-Tb (H), and ZY-Tb-BPA (I). Dry powder samples were dispersed in methanol.

Photoluminescence of samples containing BPA and nitrate anions was studied after dispersing a small amount of particles (0.2 mg/mL) in methanol. As a luminescence center, Tb³⁺ emits several spectral lines with a strongest peak at 545 nm. The excitation spectra were obtained based on this emission peak wavelength as shown in Figure 7-5. For comparison, Tb-BPA and a Y-type zeolite containing Tb and BPA were also examined. The amounts of these two samples used for luminescence test contained an equivalent amount of Tb³⁺ as that

in sample Al5Tb-BPA. The compositions of these samples are presented in Table 7-4. The estimated formula of Tb-BPA is $\text{Tb}(\text{BPA})_{2.70} \cdot 1.13\text{H}_2\text{O}$ and the molar ratio of $\text{Tb}^{3+}:\text{Al}^{3+}$ in zeolite-Y is ca. 0.50.

Table 7-4. CHN Elemental analysis and Tb cation content of $\text{Tb}(\text{BPA})_3$ salt and zeolite-Y incorporated with Tb cation and BPA.

Sample name	%Al	%Tb	%C ^c	%H ^c	%N ^c	%BPA ^d
Tb-BPA	0.00 ^a	21.37 ^a	60.36	4.26	0.00	75.90
ZY-Tb	6.79 ^b	20.00 ^b	0.00	2.77	0.05	0.00
ZY-Tb-BPA	6.89 ^b	21.10 ^b	3.30	2.79	0.09	4.15

^a Tb content was determined by ICP.

^b Al and Tb content was determined by EDS.

^c C, H, and N content was determined by CHN elemental analysis.

^d BPA content was determined based on the %C data.

Figure 7-5 shows that LDHs without Tb^{3+} (Figures 7-5A and 7-5F) do not exhibit excitation spectra (λ_{EM} 545 nm). LDH samples containing Tb^{3+} and BPA (Figures 7-5B to 7-5D) and Tb-BPA salt (Figure 7-5E) give broad excitation bands in the UV region at around 230 nm and 275 nm. In comparison, sample Al5Tb-NC only exhibits a weak excitation band at 233 nm (Figure 7-5G). Zeolite-Y which was ion exchanged with Tb^{3+} (ZY-Tb) shows a single band at 229 nm (Figure 7-5H). After incorporation of BPA (ZY-Tb-BPA), an additional band appears at 260 nm as shown in Figure 7-5I. However, it is worth noting that all samples show the absence of intra- $4f^8$ excitation lines at 380 nm ($^7F_6 \rightarrow ^5D_3$) and 490 nm ($^7F_6 \rightarrow ^5D_4$). It thus indicates that the luminescence was not due to the direct excitation into Tb^{3+} intra- $4f^8$ level, but rather, through energy transfer.³⁴² The peak at around 230 nm could be due to the excitation of the LDH host lattice as well as BPA molecules, of which the absorbed energy is subsequently transferred to the activator Tb^{3+} , as described by Blasse.³⁴³ The appearance of a broad band at the longer wavelength (275 and 260 nm) is then ascribed to the excitation of phenyl rings of BPA molecules in the interlayer. Hence, it demonstrates that

BPA anion plays an important role on the luminescence properties of the samples. A great similarity in the excitation spectra of BPA-intercalated LDHs and Tb-BPA indicates a similar electronic interaction between Tb^{3+} and BPA in these samples. A slightly different excitation spectrum is displayed by sample ZY-Tb-BPA with the peaks shifted to a shorter wavelength. The difference is possibly due to different environment and interactions surrounding Tb^{3+} ions as BPA molecules are physically adsorbed on the surface of zeolite framework.

A tailing band extending from around 300 nm to 350 nm is observed for samples Al₂₀Tb-BPA, Al₅Tb-BPA, Al₁₁Tb-BPA, and ZY-Tb-BPA, while it is not detected on Tb-BPA. A similar case was observed by Karmaoui et al. when analyzing a lamellar hybrid of lanthanides doped with Eu^{3+} and Tb^{3+} containing benzoate in the lamellae as shown by an extended excitation band from 380 nm to 480 nm.³⁴⁴ The phenomenon may demonstrate inefficiency of energy transfer between BPA molecules and Tb^{3+} ions at longer excitation wavelength.

When Tb^{3+} -containing samples were excited at 230 nm (Figure 7-6) and 275 nm (Figure 7-7), they typically generate four distinct emission peaks at 490 nm, 544 nm, 584 nm, and 619 nm that correspond to 4f orbital $^5D_4 \rightarrow ^iF_j$ transitions in the green region of the visible spectrum, where $i = 7$ and $j = 6, 5, 4, 3$, respectively. The strongest spectrum at both excitations is emitted by $\text{Tb}(\text{BPA})_3$ as shown in Figures 7-6A and 7-7A. However, the highest quantum yield is shown by sample Al₅Tb-BPA. The lower emission intensity shown by BPA-containing LDH is due to high optical density, compared to that of Tb-BPA.

Upon increasing Tb^{3+} content in LDH layers, emission intensity greatly increases until reaching a certain maximum with a molar ratio of $\text{Tb}^{3+} : \text{Al}^{3+}$ at 1.0 (Figure 7-6A to 7-6D). The same trend was observed when the samples were excited at 275 nm (Figure 7-7A to D). A higher concentration of Tb^{3+} leads to a closer distance between Tb^{3+} cations that may promote energy migration between the two identical ions, which eventually reduces the

luminescence.³⁴³ In addition, the less amount of intercalated BPA (shown in Table 7-3) in sample Al1Tb-BPA may also reduce the amount of energy transferred to Tb^{3+} cations.

On the contrary, sample Al5Tb-NC exhibits a weak emission, compared to its counterpart that contains BPA due to the lack of the interlayer photosensitizer. In addition, the formation of $\text{Tb}(\text{OH})_3$ in this sample not only reduces the concentration of Tb^{3+} cations in LDH lattice as shown by EELS image (Figure 7-3A), but also effectively quenches Tb luminescence.³⁴¹ As a comparison, ZY-Tb exhibits stronger emission than that of Al5Tb-NC, which could be due to the absence of hydroxyl group in zeolite host. The adsorption of BPA anions on the surface of zeolite framework is found to enhance the emission and enables excitation with a lower energy. Although only small amount of BPA anions was adsorbed (4.15%), it affects Tb luminescence significantly.

Figure 7-6. Emission spectra of samples NoTb-BPA (A), Al₂₀Tb-BPA (B), Al₅Tb-BPA (C), Al₁Tb-BPA (D), Tb-BPA (E), NoTb-NC (F), Al₅Tb-NC (G), ZY-Tb (H), and ZY-Tb-BPA (I). Samples were dispersed in methanol. $\lambda_{\text{EXC}} = 233$ nm for samples (A) to (G) and $\lambda_{\text{EXC}} = 229$ nm for samples (H) and (I).

Figure 7-7. Emission spectra of samples NoTb-BPA (A), Al20Tb-BPA (B), Al5Tb-BPA (C), Al1Tb-BPA (D), Tb-BPA (E), NoTb-NC (F), Al5Tb-NC (G), ZY-Tb (H), and ZY-Tb-BPA (I). Samples were dispersed in methanol. $\lambda_{\text{EXC}} = 275$ nm for samples (A) to (G) and $\lambda_{\text{EXC}} = 260$ nm for samples (H) and (I).

Macromolecules with aromatic rings are often used as ligands to form complex compounds with lanthanides to improve their luminescence by reducing non-radiative deactivation as well as to shift the excitation to lower energy via energy transfer from ligand centers to the lanthanide ions, the so-called antenna effect. It has been subjected to several reviews regarding the use of ligands as sensitizers.^{333, 345-346} As a simple molecule, BPA consists of one biphenyl group. The work reported by Karmaoui et al. utilized 4-biphenylmethanol to synthesize lanthanide-biphenolate lamellar hybrid.³⁴² Having a similar structure as BPA, biphenolate facilitates excitation at a lower energy and at the same time

increases luminescence via energy transfer. Therefore, it is believed that 4-biphenylacetate exhibits similar phenomena.

In order to understand the mechanism of energy transfer between BPA and Tb^{3+} cation, the emission spectra were examined by varying the excitation wavelength from 230 nm to 400 nm (Figure 7-8). Figure 7-8A shows that excitation of NoTb-BPA results in broad emission bands extending from 380 nm to 600 nm and centered at around 430 nm, which is due to the emission of BPA. The intensity decreases gradually when the excitation wavelength is varied from 233 nm to 260 nm. A sudden increase of the intensity at 275 nm is then observed, after which it diminishes with increased excitation wavelength.

When a small amount of Tb^{3+} was incorporated (Al20Tb-BPA), the emissions due to BPA are still strongly evidenced, which superimposed with $^5D_4 \rightarrow ^7F_{6-3}$ transitions of Tb^{3+} (Figure 7-8B). As the content of Tb^{3+} increases (Figures 7-8C and D), the intensity of BPA emission band gradually decreases. On the other hand, no emission arising from BPA could be observed when Tb-BPA was excited (Figure 7-8E). Based on such phenomena, the mechanism of the energy transfer from BPA to Tb^{3+} center can be proposed. According to Dieke diagram, the energy gap between the excited state 5D_4 and the ground state 7F_6 of Tb^{3+} is about 21000 cm^{-1} , which is equivalent to the photon energy at a wavelength of 470 nm.³⁴⁷ Noting that the emission of BPA anion has a slightly higher energy than the excited state 5D_4 of Tb^{3+} , it may yield good resonance that enables an efficient transfer. The observation on the decreasing intensity of BPA emission bands with increasing Tb^{3+} concentration confirms the proposed mechanism. A higher content of Tb^{3+} results in more energy transfer from BPA anion, which eventually leads to the diminishing signal from BPA anion. The energy transfer in Tb-BPA is more efficient due to the minimum equilibrium distance between BPA and Tb^{3+} . Therefore, the absence of BPA emission and stronger luminescence of Tb^{3+} can be observed (Figure 7-8E). Accordingly, sample ZY-Tb-BPA demonstrates a similar pattern while ZY-Tb only exhibits luminescence by excitation at 229 nm. Besides acting as a sensitizer, the

presence of hydrophobic BPA also minimizes the absorption of water molecules by LDHs and thus reduces the quenching effect.

Figure 7-8. Emission spectra of samples NoTb-BPA (A), Al₂₀Tb-BPA (B), Al₅Tb-BPA (C), Al₁Tb-BPA (D), Tb-BPA (E), NoTb-NC (F), Al₅Tb-NC (G), ZY-Tb (H), and ZY-Tb-BPA (I) obtained by varying excitation wavelength from 230-400 nm. Samples were dispersed in methanol.

Figure 7-9. Photoluminescent pictures of samples NoTb-BPA (A), Al₂₀Tb-BPA (B), Al₁₁Tb-BPA (C), Al₅Tb-BPA (D), Tb-BPA (E), NoTb-NC (F), Al₅Tb-NC (G), ZY-Tb (H), and ZY-Tb-BPA (I). Samples were dispersed in methanol. $\lambda_{\text{EXC}} = 233$ nm for samples (A) to (G) and $\lambda_{\text{EXC}} = 229$ nm for samples (H) and (I).

Figure 7-10. Photoluminescent pictures of samples NoTb-BPA (A), Al₂₀Tb-BPA (B), Al₁₁Tb-BPA (C), Al₅Tb-BPA (D), Tb-BPA (E), NoTb-NC (F), Al₅Tb-NC (G), ZY-Tb (H), and ZY-Tb-BPA (I). Samples were dispersed in methanol. $\lambda_{\text{EXC}} = 275$ nm for samples (A) to (G) and $\lambda_{\text{EXC}} = 260$ nm for samples (H) and (I).

Figures 7-9 and 7-10 present the fluorescent pictures of the samples excited at around 230 nm and 270 nm, respectively. Overall, the excitation at a longer wavelength results in brighter fluorescence. It is possible that excitation at a shorter wavelength is followed by non-radiative deactivation, thus leaving a smaller fraction of energy to be transferred to Tb^{3+} centre.³⁴³

Sample NoTb-BPA emits blue color, which corresponds to emission of BPA molecules at around 430 nm. Upon increasing Tb^{3+} content, the color gradually becomes green due to the dominant emission from Tb^{3+} . Herewith, the emitted color can be tuned by varying the composition of Tb^{3+} and the type of intercalated sensitizer by comparing the colors displayed from various samples. Nonetheless, a weak blue emission is given by sample NoTb-NC upon excitation at 275 nm despite the absence of BPA and Tb^{3+} cation (Figure 7-10F). Some evidence on the “fluorescent” property of LDHs at 430 nm were also observed elsewhere.³⁴⁸⁻³⁴⁹ It is possibly due to the scattering of the incident beam by hydroxyl groups as reported by Jeziorowski et al.³⁵⁰ On the other hand, sample Al5Tb-NC shows a faint green color. Sample ZY-Tb also emits very faint green emission and the intensity is enhanced after incorporation of BPA. The weak intensity shown by ZY-Tb-BPA is due to a smaller amount of BPA incorporated in this sample (Table 7-4) compared to those in BPA-intercalated LDHs.

Figure 7-11 presents the time-domain lifetime measurement at room temperature with excitation at 233 nm and 275 nm. The measurement was performed for dilute suspensions of the samples in methanol. The curved characteristics of the logarithmic decay graphs indicate that they can not be fitted by a single exponential decay, but rather, double exponential equation as follows with $r^2 > 0.9995$.²¹⁴

$$I(t) = Po + A_1 \exp(-t / \tau_1) + A_2 \exp(-t / \tau_2) \quad (7-2)$$

where Po is the Poisson noise, τ_1 and τ_2 are the lifetimes for each component, A_1 and A_2 are the intensity amplitudes at $t = 0$. The average lifetime τ_{avg} is given by

$$\tau_{avg} = \frac{A_1\tau_1^2 + A_2\tau_2^2}{A_1\tau_1 + A_2\tau_2} \quad (7-3)$$

Figure 7-11. Luminescence decay curves of Tb^{3+} emission at 545 nm obtained by excitation of suspensions in methanol at 233 nm (A) and 275 nm (B).

The multiexponential decays displayed by the samples could demonstrate complex phenomena occurring concurrently. The occurrence of energy transfer between BPA anions and Tb^{3+} cations, as well as the quenching by LDH hydroxide layers and the solvent (methanol) may together affect the fluorescence decay profile.²¹⁴ Furthermore, different

crystallinity of LDH layers could influence the crystal field which eventually affects the emission property and the lifetime.³⁴³

Table 7-5. Lifetime of suspended samples at different excitation wavelengths with normalized pre-exponential values ($Po + A_1 + A_2 = 1$). Emission wavelength was at 545 nm.

Suspension Sample	Excitation WL (nm)	Poisson noise (Po)	A_1	τ_1 (ms)	A_2	τ_2 (ms)	τ_{avg} (ms)
Al1Tb-BPA	234	0.000	0.024	5.748	0.985	1.279	1.71
	275	0.002	0.042	0.345	0.964	1.337	1.33
Al5Tb-BPA	233	0.000	0.160	2.362	0.826	1.103	1.47
	275	0.002	0.083	0.309	0.925	1.293	1.27
Al20Tb-BPA	233	0.029	0.203	0.504	0.781	1.326	1.25
	275	0.009	0.163	0.326	0.833	1.263	1.22
Tb-BPA	233	0.003	0.053	0.215	0.952	1.195	1.19
	275	0.000	0.017	0.061	0.993	1.239	1.24
Al5Tb-NC	235	0.141	0.218	0.353	0.636	1.272	1.19
ZY-Tb	227	0.052	0.088	0.260	0.879	1.149	1.13
ZY-Tb-BPA	228	0.039	0.086	0.343	0.874	1.188	1.16
	262	0.016	0.110	0.439	0.892	1.109	1.08

Table 7-5 summarizes the lifetime values for all samples. In general, it is observed that the emission intensity is fractioned into two amplitudes with different magnitude, which are represented by pre-exponential values A_1 and A_2 , each of which corresponds to one of decay times τ_1 and τ_2 , respectively. The presence of two amplitudes may also indicate the contribution of more than one fluorophores, which is in our case, Tb^{3+} cations and BPA anions. The Poisson noise indicates a random fluctuation of the received emission signal and its magnitude becomes more significant with decreasing amount of received signal, such as the one observed for sample Al5Tb-NC. Apart from sample Al5Tb-NC, ZY-Tb, and ZY-Tb-BPA, the rest of samples exhibit small values of Poisson noise, which could minimize the error of the resolved two decay times.³⁵¹

It can generally be observed from Table 7-5 that the larger fraction of the intensity consistently has a decay time ranging from 1.1 to 1.3 ms, while the smaller one corresponds to

a short decay time (0.06 to 0.5 ms). The results hence support the phenomena of energy transfer. An efficient energy transfer from BPA molecules that occurs fast enough results in a short lifetime (0.061 ms) and small amplitude (0.017), as displayed by Tb-BPA upon excitation at 275 nm. As a comparison, an inefficient transfer occurs by excitation of the same sample at 233 nm, which results in a longer lifetime (0.215 ms) and larger amplitude (0.053). Therefore, by comparing the lifetime and the magnitude of the samples excited at those two different wavelengths, it can be concluded that energy transfer occurs more efficiently at 275 nm, which contributes to the brightness of the emission at the stipulated wavelength.

Table 7-6. Quantum yields (Q) of samples suspended in methanol.

Suspension sample	Quantum yield (Q)	
	$\lambda_{\text{EXC}} \sim 230 \text{ nm}$	$\lambda_{\text{EXC}} \sim 275 \text{ nm}$
Al20Tb-BPA	0.1356	0.1507
Al5Tb-BPA	0.2196	0.2162
Al1Tb-BPA	0.1539	0.1404
Al5Tb-NC	0.0031	0.0024
Tb-BPA	0.0926	0.0882
ZY-Tb	0.0072	0.0009
ZY-Tb-BPA	0.0129	0.0098

Quantum yields of samples are presented in Table 7-6. A small amount of powdered sample was suspended in methanol and a dilute cresyl violet solution (10^{-7} M) in methanol was used as a reference. Since the optical density of the suspension is much larger than 0.05, the correction to the emission intensity needs to be done by applying eq. 7-2. Al5Tb-BPA shows the highest value, which is around 22%, while the lowest one belongs to Al5Tb-NC ($Q \sim 0.2\%$). Upon increasing Tb^{3+} : Al^{3+} ratio from 0.05 to 0.2, quantum yield increases then decreases when the ratio was increased to 1.0. This is due to non-radiative energy migration among densely populated Tb^{3+} ions in the LDH matrix as described earlier. The effect of

BPA in increasing quantum yield is also observed by comparing samples ZY-Tb and ZYTb-BPA, which the latter (~1.2%) has higher value than the former (~0.7%). For comparison, ZY-Tb-BPA has a quantum yield similar to those obtained by Wada et al. for Tb³⁺-doped zeolite with 4-acetylbiphenyl as the photosensitizer.³³⁴ Overall, the estimated quantum yields of our samples are comparable to those reported for other lanthanides doped in organic-inorganic hybrid materials.³⁴⁴ Finally, it is worth noting that among the lanthanides, Tb³⁺ ion exhibits a relatively large energy gap between its emissive and ground states. Although hydroxyl groups are recognized as the efficient quenchers to lanthanide luminescence, the extent of the quenching is inversely proportional to this energy gap. Quenching via nonradiative energy transfer to the crystal lattice becomes less efficient when a large quantum of energy must be dissipated. As a result, the hydroxyl groups of the LDH lattice seem not to cause a significant quenching effect to Tb³⁺ luminescence.

7.3.3 Photoluminescence properties of Tb(III)-doped LDHs intercalated with other types of drug compounds

Besides BPA, other types of drug compounds such as 5-fluorouracil (FU, anticancer drug), valproic acid (VA, anticonvulsant), and folic acid (FA, model drug for anticancer methotrexate) were intercalated into Tb³⁺-doped LDHs with Tb³⁺: Al³⁺ molar ratio of 0.20, which are denoted as Al5Tb-FU, Al5Tb-VA, and Al5Tb-FA, respectively. The chemical structures of various drug compounds are shown in Figure 7-12. The effects of different chemical structure of the intercalated drug compound on the fluorescence emissions and the energy transfer involved are presented in the remaining part of this chapter.

Figure 7-13 displays the XRD patterns of LDHs containing different drug compounds. It is observed that different compound interacts and orientates differently in the interlayer, therefore resulting in different basal spacing, crystallinity and brucite-like layer stacking. Basal spacing of Al5Tb-VA is 19.5 Å, which is slightly larger compared to that reported by

Khan et. al. (18.7 Å).¹⁰⁰ Meanwhile, the (003) peak of Al5Tb-FA and Al5Tb-FU is hard to define as it is very broad with 2θ onsets from 3° to 9° , indicating poor stacking of the brucite-like layers and low crystallinity. The arrangement of the cations in the brucite-like layers is also very poor as (110) peaks for both samples are barely recognizable. Therefore, it can be advised that FA and Fu anions are mainly adsorbed on the irregular LDH particles.

Figure 7-12. Chemical structures of 4-biphenylacetic acid (A), valproic acid (B), 5-fluorouracil (C), and folic acid (D).

Figure 7-13. XRD patterns of Al5Tb-BPA (A), Al5Tb-VA (B), Al5Tb-FA (C), and Al5Tb-FU (D).

FTIR spectra are shown in Figure 7-14. Samples Al5Tb-FA and Al5Tb-FU exhibit very weak M—O absorption band as shown in Figures 7-14B and 7-14C, respectively, compared to a strong one exhibited by Al5Tb-VA (Figure 7-14D). Such observations are consistent with the poor resolution of (110) peak observed from the XRD pattern for samples Al5Tb-FA and Al5Tb-FU. Nevertheless, the presence of the drug molecules is strongly evidenced on the infrared spectra. For sample Al5Tb-FU, absorption bands at 1625, 1650, and 1686 cm^{-1} are attributed to C=C, C=N, and C=O bonds of FU anion, respectively. The absorption at 1400 cm^{-1} can be attributed to the vibration of the multi-substituted pyrimidine compound, and the strong absorption band at 1281 and 1168 cm^{-1} can be assigned to the vibration of C—O and C—N, respectively.²⁰⁰ These results hence indicate that 5-fluorouracil is negatively charged as one of the oxygen atoms is ionized. In addition to the drug anions, the presence of nitrate anion is evidenced by a sharp peak at 1384 cm^{-1} . For sample Al5Tb-FA, the stretching vibration of C=O from —COOH at 1694 cm^{-1} is not observed, verifying that it is in the ionized form. Absorption at 1407 cm^{-1} is attributed to C=C stretching vibration mode in the backbone of aromatic rings,³⁵² a band at 1606 cm^{-1} is assigned to bending vibration of —NH₂ group, and a strong band at 1511 cm^{-1} designates the phenyl ring.⁵⁹ Lastly, the intercalation of valproic acid (Figure 7-14D) gives absorption bands at 2850-2970 cm^{-1} (C—C and C—H vibration mode), 1410, and 1535 cm^{-1} (symmetric and antisymmetric —COO[−], respectively). However, nitrate anion is also present in the interlayer of this sample, as a very strong peak at 1384 cm^{-1} is observed.

Figure 7-14. FTIR spectra of Al5Tb-BPA (A), Al5Tb-FA (B), Al5Tb-FU (C), and Al5Tb-VA (D).

The morphology of samples containing different drugs is observed with TEM technique and is displayed in Figure 7-15. All samples except Al5Tb-VA exhibit irregularly-shaped and aggregated particles that are folded and entangled. Individual plate-like particles can be observed for sample Al5Tb-VA.

The compositions of cations in the samples are obtained by ICP and are presented in Table 7-7. It is found that Tb^{3+} : Al^{3+} molar ratio is relatively the same, ranging from 4.0-4.5. Apart from sample Al5Tb-FU, the molar ratio of M^{2+} : M^{3+} is around 2.0, which is consistent with that in precursor solutions. A low content of Mg^{2+} is observed for sample Al5Tb-FU, which leads to M^{2+} : M^{3+} of 1.14. Mg^{2+} cations may have partially leached out from LDH lattice during aging, due to a significantly higher solubility of Mg^{2+} compared to other cations.¹¹⁷ It therefore affects the atomic arrangement in LDH octahedral lattice and further causes poor crystallinity and morphology.

Figure 7-15. TEM images of Al5Tb-BPA (A), Al5Tb-FA (B), Al5Tb-FU (C), and Al5Tb-VA (D). The scale bars indicate 50 nm.

Table 7-7. Weight percentages of cations in samples containing different drug compounds.

Sample name	Mg : (Al+Tb) molar ratio	Tb : Al molar ratio	%Mg	%Al	%Tb
Al5Tb-BPA	2.17	4.36	10.85	4.50	6.89
Al5Tb-FA	2.05	4.17	9.48	4.14	5.84
Al5Tb-FU	1.14	4.57	11.66	9.28	11.96
Al5Tb-VA	2.24	4.48	16.50	6.70	8.80

Figure 7-16. Excitation spectra, $\lambda_{EM} = 545$ nm (A) and emission spectra (B) of Al5Tb-FU, Al5Tb-FA, and Al5Tb-VA.

Figure 7-16 displays the excitation (A) and emission spectra (B) of samples Al5Tb-FU, Al5Tb-FA, and Al5Tb-VA. The excitation spectrum of Al5Tb-FU consists of a broad band peaked at 295 nm in addition to the one at 235 nm, while Al5Tb-VA has only a single band at 235 nm. In contrast, sample Al5Tb-FA is not luminescence active as there is no excitation band observed. Upon excitation at the maximum wavelengths, samples Al5Tb-FU and Al5Tb-VA exhibit typical $^5D_4 \rightarrow ^7F_j$ emissions of Tb^{3+} with distinctive peaks at around 490, 545, 585, and 620 nm. However, their intensities are considerably lower than that of Al5Tb-BPA. On the other hand, sample Al5Tb-FA emits a broad emission band centered around 450 nm and the emission of Tb^{3+} is not observed. Figure 7-17 shows the luminescence pictures of samples excited at the corresponding wavelength. Sample Al5Tb-FU shows weak green emission upon excitation at 235 nm (Figures 7-17A) and the brightness increases significantly upon excitation at 295 nm (Figure 7-17B). It indicates an effective energy transfer between 5-fluorouracil and Tb^{3+} ions. Meanwhile, sample Al5Tb-VA also exhibits a weak green emission (Figure 7-17D). Lastly, a faint blue emission is displayed by sample Al5Tb-FA (Figure 7-17C), corresponding to its emission spectrum in the blue region.

Figure 7-17. Photoluminescence pictures of Al5Tb-FU, $\lambda_{\text{EXC}} = 235$ nm (A), Al5Tb-FU, $\lambda_{\text{EXC}} = 295$ nm (B), Al5Tb-FA, $\lambda_{\text{EXC}} = 235$ nm (C), and Al5Tb-VA, $\lambda_{\text{EXC}} = 235$ nm (D).

The results thus demonstrate that various intercalated drug m^{353} olecules affect luminescence properties differently. Luminescence property of organic compounds results from the transfer of the valence π -electrons to the antibonding π^* -orbital, which are normally possessed by conjugated compounds.³⁵⁴ Hence, the nature of electronic transitions is dependent on the molecular structure. One main factor that makes a compound highly fluorescent is the rigidity of the structure. Compared to a straight chain, aromatic compounds exhibit a rigid structure, which minimizes the non-radiative energy losses due to molecular vibration and rotation.³⁵⁴ Among drug compounds that have been used, 4-biphenylacetic acid and 5-fluorouracil fit the criteria well. It is thus well expected for these compounds to enhance the luminescence. However, 4-biphenylacetic acid exhibits a better performance compared to 5-fluorouracil. The latter consists of oxygen atoms that may promote quenching. In addition, the presence of $-\text{NH}$ and $-\text{C}=\text{O}$ groups may act as good oscillators that absorb a substantial amount of energy although they are less effective than $-\text{OH}$.^{346, 355} Meanwhile, folic acid consists of few aromatic rings that are embedded in the carbon chain. This structure is therefore more flexible and absorbs more energy. The presence of oxygen atoms, $-\text{NH}$, $-\text{C}=\text{O}$, and aliphatic $-\text{CH}$ group also contributes to the non-radiative deactivation of the molecule and makes it non-fluorescent. Lastly, the short-branched aliphatic chain of valproic acid shows no fluorescence. Hence, the emission from Tb^{3+} is most likely due to energy transfer from the excited LDH lattice.

7.4 Conclusion

Incorporation of Tb^{3+} cations into the brucite-like layers of LDH was successfully achieved using a simple coprecipitation technique in the presence of various drug compounds. Detailed characterizations confirmed a homogeneous distribution of Tb^{3+} cations in the octahedral lattice of LDH layers despite its relatively large ionic size. A high content of Tb^{3+} up to around 19 wt% was obtained without promoting phase segregation of Tb^{3+} . The investigation on luminescence properties suggested that there is energy transfer from the intercalated molecules to Tb^{3+} centers based on the appearance of excitation at a longer wavelength, which further enhances the luminescence. In addition, the emission color can be tuned by changing the excitation wavelength and use of the appropriate interlayer sensitizers. Therefore, it opens new opportunity in creating a novel photoluminescent drug-containing LDHs with multifunctionality.

CHAPTER 8 CONCLUDING REMARKS AND FUTURE WORK

8.1 Concluding Remarks

The works presented in the preceding chapters are mainly concerned with MgAl-LDHs containing various types of drug compounds in order to prepare organic-inorganic LDH hybrids as drug nanocarriers. A simple coprecipitation technique was used during syntheses of materials followed with post-synthesis treatment of the resulting mixture either at atmospheric conditions or at elevated pressures *via* solvothermal aging. In addition, different synthesis media and additives have been applied to obtain materials with different size, morphology, and physicochemical properties. Thorough characterizations and analyses were performed to study the properties of the obtained products as well as to investigate in details the mechanism involved during the synthesis. Several important results and findings are summarized as follows.

- 1) The use of different media, such as ethylene glycol and water, and hydrothermal treatment in the synthesis of ibuprofen-intercalated LDHs as model drug is able to tune LDH particle size and to alter the LDH surface properties. Under hydrothermal conditions, synthesis in the mixture of ethylene glycol and water results in relatively uniform platelets of around 500 μm . It also promotes the formation of *c*-oriented dense powder upon drying, which is attributed to the preferential surface-to-surface and edge-to-edge aggregations of the large, regularly shaped, and crystalline drug-LDH platelets. The release rate of ibuprofen is considerably slower from the oriented powder sample than that from the loosely aggregated powder.
- 2) Self-assembly of LDH nanoparticles to form three-dimensional coral-like MgAl LDH microspheres of around 1 μm in diameter was achieved via a simple and effective solvothermal method in a nonaqueous polar solvent/surfactant system of EG/methanol/DS. It was found that the rates of nucleation and crystallization of LDHs were effectively

reduced in EG/methanol solvent mixture with 1:1 volumetric ratio due to the solvation effects. Through a self-assembly process, LDH microspheres consisting of the constituent nanoparticles can be formed at 100 °C. At a higher temperature of 150 °C, the constituent nanoparticles recrystallized to thin nanoplatelets of 3-4 monolayers, thereby resulting in a coral-like hierarchical structure. The ion exchange of surfactant with drug anions, such as 4-biphenylacetate, ibuprofen and naproxen anions was proven to retain the morphologies of the microspheres.

- 3) Deposition of LDH nanocrystals and formation of closely packed LDH films onto the surface of CNS results in hollow structure after removal of CNS core via calcination. The resulting hollow oxides show a uniform size of about 300-400 nm. It is identified that a key requirement to achieve high quality hollow nanospheres is the use of a stable colloidal suspension of well-dispersed LDH nanocrystals as the building units. The shell wall of the nanospheres was found to be robust enough to withstand the mechanical stirring and ultrasonication. Upon reconstruction process in the presence of ibuprofen as a model drug, the hollow structure of the resulting drug-LDH product was well maintained by carefully tuning the reconstruction conditions, such as the amount of water, aging temperature, and time, in order to control the crystallization rate of LDH nanoparticles.
- 4) Incorporation of Tb^{3+} cations into the hydroxide layers of LDH was carried out using a simple coprecipitation technique in the presence of a drug compound, 4-biphenylacetic acid (BPA). Detailed characterizations confirmed a homogeneous distribution of Tb^{3+} cations in the octahedral lattice of LDH layers despite the relatively large size of terbium ions, and a high content of Tb^{3+} up to around 20 wt% was achieved without promoting phase segregation of Tb. The investigation on luminescence properties suggested that there was energy transfer from BPA molecules to Tb^{3+} centers by the appearance of excitation at a longer wavelength, which further enhances the luminescence. In addition, the emission color can be tuned by changing the excitation wavelength.

8.2 Future Work

Despite abundant literatures available regarding the development of LDHs, a large amount of research work needs to be done to produce highly functionalized materials for pharmaceutical applications. Below are some suggestions for the extension of the current work.

1. As drying process may affect the aggregation of the resulting powder, a better control of heating rate and drying period should be further investigated.
2. Inclusion of nanomaterials of other functionalities such as magnetic nanoparticles or quantum nanodots into the interior space of the LDH hollow nanospheres. By doing so, multifunctional drug-containing LDHs can be obtained. The novel materials will therefore possess magnetic and photoluminescence properties, which could be useful in pharmaceutical field as they can be used as non-invasive diagnostic tools.
3. Surface coating of drug-containing LDH nanoparticles with poly(ethylene glycol) (PEG) in order to prolong their circulation in the blood course. The use of PEG as a stealthing agent has been widely used on other systems to help increase the circulation time of nano- and microparticles. The PEG layer prevents the adsorption of plasma proteins (opsonins) that triggers phagocytosis by reticulo-endothelial system (RES). It is believed that the novel PEG-modified LDHs will greatly improve the potential use of LDH nanoparticles as intravenous drug carriers.
4. Introduction of other lanthanide ion together with Tb^{3+} , such as Eu^{3+} that emits red emission, into the crystal lattice of LDH hydroxide layers in order to adjust the emission color of the obtained material by changing the excitation wavelength. In addition, the dispersion of LDH particles must also be improved to enhance their use intravenously.
5. Lanthanides are usually excited within UV range, which damages human tissues, thus their clinically use is restricted. Co-doping of Tb^{3+} or Er^{3+} with Yb^{3+} may be able to promote up-conversion. It is a mechanism of using longer excitation wavelength to

generate shorter emission wavelength. One common phenomenon is excitation with infrared light to generate emissions in the visible light region, which will be safe for *in vivo* applications.

6. Along with the advancement of cancer treatment, targeted drug nanocarriers to the affected cells become more desirable as it will increase the effectiveness of therapeutics performance as well as reduce negative side effects on the healthy cells. As a potential drug carrier, the surface of LDH nanoparticles can be modified by attaching targeting ligands, such as antibodies, peptides, and folate that are recognizable by the over-expressed receptors on tumor cells. To achieve such performance, surface functionalization of LDH particles must be further explored.

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