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GRADUATE UNIVERSITY SCIENCE AND TECHNOLOGY

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SYNTHESIS OF ZnAl-LAYERED DOUBLE HYDROXIDE NANOMATERIALS FOR DRUG DELIVERY TARGETING TREATMENT OF INFECTED WOUNDS

SUMMARY OF DISSERTATION ON SCIENCES OF MATTER

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INTRODUCTION

1. Background and Rationale

Wound infection arises when bacteria penetrate the skin barrier, causing inflammation, pain, and potentially life-threatening complications such as sepsis. Advances in understanding wound pathophysiology have fostered the development of controlled or sustained drug delivery systems, in which therapeutic agents are released in a time-regulated manner to maximize efficacy, reduce dosing frequency, and minimize adverse effects.

Nanocarrier-based controlled delivery has gained considerable attention. Among inorganic layered materials, layered double hydroxides (LDHs) are promising drug carriers owing to their large surface area, interlayer anion exchange capacity, drug-protective intercalation, hydration ability, tunable release kinetics, pH responsiveness, high charge density, low cytotoxicity, and excellent biocompatibility. Although LDHs have been explored mainly for oral administration, their potential in topical drug delivery is under-recognized. In this study, ZnAl-LDH was selected as the carrier of antibiotics and anti-inflammatory drugs due to the antibacterial properties of zinc and the relatively low cytotoxicity of aluminum.

Topical antibiotic therapy offers distinct advantages: reducing systemic misuse, lowering effective doses, and minimizing systemic side effects. Yet, carrier toxicity remains a major barrier. LDHs, with their low toxicity and biocompatibility, present a promising alternative for delivering antibacterial and anti-inflammatory drugs directly to the wound site, thereby accelerating healing and reducing inflammation. Notably, no study has simultaneously investigated LDH carriers for both antibiotic and anti-inflammatory agents in the context of infected wound management.

Another critical issue is drug loading efficiency. Conventional one-variable-at-a-time optimization is inefficient. Response surface methodology (RSM), particularly central composite design (CCD), provides a robust statistical approach to model the relationships between process variables and responses, reducing experimental time and cost while improving optimization outcomes.

2. Research objectives

The objective of the research entitled *"Synthesis of ZnAl-LDH layered double hydroxide nanomaterials for drug delivery targeting treatment of infected wounds"*, aims to develop ZnAl-LDH composites capable of controlled release of antibiotics and anti-inflammatory drugs at appropriate dosages and timings, with the dual goals of bacterial eradication and inflammation suppression. Optimization of drug loading efficiency for levofloxacin, ciprofloxacin, salicylic acid, and ibuprofen into ZnAl-LDH is performed using CCD-RSM.

3. Research contents

Synthesis and characterization of ZnAl-LDH (TGA, XRD, FTIR, SEM), antibacterial testing against *Escherichia coli* and *Staphylococcus aureus*, and cytotoxicity evaluation on RAW264.7 cells.

Optimization of antibiotic (LEV, CIP) and anti-inflammatory drug (SAL, IBU) loading using CCD-RSM.

Characterization of DRUG–LDH composites (LEV-LDH, CIP-LDH, SAL-LDH, IBU-LDH) by TGA, XRD, FTIR, and SEM.

Study of controlled release profiles, release kinetics of the DRUG-LDH.

Assessment of antibacterial activity (*E.coli* and *S.aureus*) and cytotoxicity against RAW264.7 cells.

4. Scientific and practical value

The results provide a scientific foundation for the development of solid-state LDH-based carriers for controlled drug delivery in wound management.

The drug–LDH formulations (LEV-LDH, CIP-LDH, SAL-LDH, IBU-LDH) demonstrate strong antibacterial performance and low cytotoxicity, suggesting their potential applications in infected wound care and therapy.

5. New contributions of the thesis

Multifunctional ZnAl-LDH nanomaterials were successfully synthesized through a straightforward process, with optimized conditions for loading antibiotics (LEV, CIP) and anti-inflammatory drugs (SAL, IBU) established using RSM-CCD.

The unique transport and controlled drug-release properties of ZnAl-LDH are expected to enhance the treatment of infected wounds by sustaining therapeutic drug concentrations at the wound site for up to 12 hours, while simultaneously preventing recurrence or progression of inflammation and bacterial growth.

As a potential candidate for topical therapy, ZnAl-LDH may lower the required dosage for pathogen elimination, thereby minimizing systemic exposure. Furthermore, its capacity to potentiate antibiotic effectiveness provides a potential strategy to overcome bacterial resistance, addressing a critical challenge in modern healthcare.

CHAPTER 1. OVERVIEW

1.1. Overview of wounds and factors contributing to delayed healing

Skin, the body's largest organ, acts as the first barrier against external damage. Skin injury initiates complex cellular responses that regenerate epidermis and dermis, restoring damaged tissue. Wound healing, a natural physiological process, is vital for tissue repair and protection. The efficiency of healing is influenced not only by intrinsic mechanisms but also by extrinsic factors including diabetes, vascular disorders, bacterial infections, prolonged inflammation, and lifestyle. Infection prolongs inflammation, delays healing, and may cause pus, ulceration, or systemic complications. Common bacterial species isolated from wound surfaces include *Staphylococcus aureus* (37%), *Pseudomonas aeruginosa* (17%), *Proteus mirabilis* (10%), *Escherichia coli* (6%), and *Corynebacterium* spp. (5%). However, conventional antibiotic or anti-inflammatory therapy lacks dosage control and is often associated with adverse effects. Consequently, advanced drug delivery platforms are being developed to overcome such limitations and enhance infected wound therapy, with LDHs considered one of the most promising inorganic carriers.

1.2. Layer double hydroxide

Layered double hydroxides (LDH) are two-dimensional anionic clays at the nanoscale characterized by the general formula:

$$[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}[(A^{n-})_{x/n}.mH_2O]^{x-}$$

Where, M^{2+}: divalent cations (Cu^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+} …)

M^{3+}: trivalent cations (Al^{3+}, Fe^{3+}, Ga^{3+}, Cr^{3+} …)

A^{n-}: interlayer anions (Cl^-, Br^- , NO_3^-, I^- , OH^-, SO_4^{2-}, ...)

x: molar ratio $M^{3+}/(M^{2+} + M^{3+})$, typically 0,2–0,33.

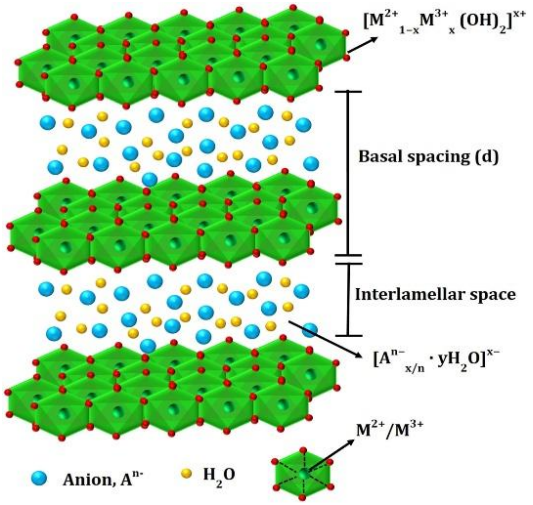


Figure 1.1. *Structure of layered double hydroxide*

LDH may be prepared using multiple synthetic routes, including co-precipitation, hydrothermal treatment, ion exchange, salt–oxide conversion, urea hydrolysis, sol–gel processing, mechanochemistry, ...

LDH possess remarkable features, including high adsorption ability, biodegradability, excellent biocompatibility, large surface-to-volume ratio, and ease of exfoliation. LDH also show pH sensitivity and a memory effect. LDH are synthesized via simple, low-cost, tunable methods. Consequently, LDHs are investigated across diverse applications, including adsorption, catalysis, separation, energy storage (supercapacitors), biomedical systems, sensors, and environmental remediation.

Based on the specific properties of LDHs, incorporating antibiotics (levofloxacin, ciprofloxacin) and anti-inflammatory drugs (salicylic acid, ibuprofen) into LDHs not only enables controlled drug release but also reduces adverse effects.

1.3. Drug loading optimization by RSM

1.3.1. Drug loading optimization objective

The objective of optimizing drug loading is to maximize the incorporation of therapeutic molecules within the molecular framework or interlayer

galleries of LDHs, ensuring material quality, structural stability, and controlled-release capability. Such optimization is intended to enhance delivery efficiency, improve bioavailability, lower required dosages, and reduce adverse drug effects.

1.3.2. Response surface methodology

Response surface methodology (RSM) integrates mathematical and statistical tools that have been successfully applied to process design and optimization. RSM enables visualization of interactions among independent variables influencing a target function, using limited experiments. RSM identifies optimal factor levels for maximizing a target response within the studied domain. In biomedicine, RSM has been extensively used for optimizing drug loading and associated parameters.

Central composite design CCD represents a common design approach used in RSM. In CCD, central points represent the midpoint of the experimental domain. Factorial points are denoted at -1 and +1 levels, and axial points are symmetrically distributed along coordinate axes around the center. Compared with full three-level factorial designs, CCD is more efficient, reducing the number of experiments without compromising results. Thus, CCD is one of the most widely accepted experimental designs for quadratic modeling.

1.4. Kinetic models of drug release

Drug release kinetics depend on matrix composition, morphology, synthesis method, and dissolution medium. Models such as first-order, Higuchi, Korsmeyer–Peppas, and parabolic diffusion describe drug release mechanisms.

CHAPTER 2. EXPERIMENTS

2.1. Synthesis of layered double hydroxide ZnAl-LDH

ZnAl-LDH was synthesized by the co-precipitation.

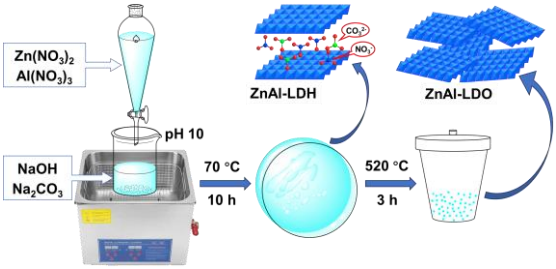


Figure 2.1. *Schematic diagram of ZnAl-LDH synthesis*

2.2. Optimization of Drug Loading into ZnAl-LDH by RSM-CCD

Table 2.1. *Independent variables, experimental range, and levels for CCD (the amount of ZnAl-LDH kept constant at 1.0 g in all experiments)*

Coded values	Variable and range			
	Temperature X_1 (°C)	Time X_2 (h)	Amount of drug X_3 (g)	
			CIP/LEV/SAL	IBU
High (+1)	75.0	19.0	1.00	0.500
Medium (0)	65.0	17.5	0.75	0.375
Low (-1)	55.0	16.0	0.50	0.250

Experiment on drug loading into ZnAl-LDH

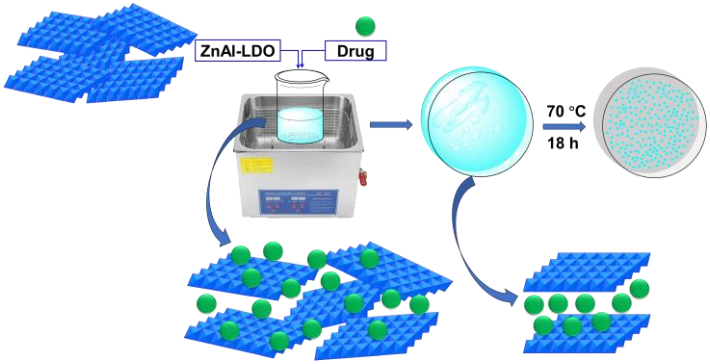


Figure 2.2. *Procedure for loading LEV/CIP/SAL/IBU into ZnAl-LDH*

2.3. Characteristics of ZnAl-LDH and DRUG-LDH

ZnAl-LDH and DRUG-LDH were analyzed for composition and structural properties through XRD, FTIR, SEM, DLS, and TGA.

2.4. Controlled drug release study

Drug release was tested by dialysis in phosphate buffered saline (PBS) at pH 5.8 and 7.4.

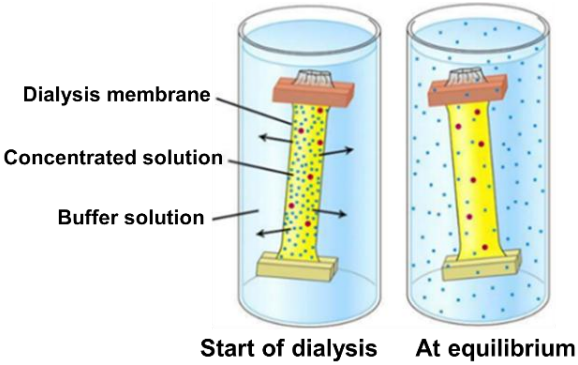


Figure 2.3. *Drug release experiment by dialysis method*

Cumulative release of drug was calculated according to the following formula:

$$\text{Amount of Cumulative release (mg/g)} = \frac{C_t \times V + \sum_{t-0}^{n-1} C_t \times V_t}{m}$$

$$\%\ \text{Cumulative release of drug} = \frac{\text{Amount of Cumulative} \times 100}{m_0}$$

where, V : total solution volume of release (50 mL)

V_t : volume of release media removed every time (1 mL)

C_t : amount of drug in release media removed every time release media removed every time (mg)

m : amount of DRUG-LDH (0.1 g)

m_0 : amount of drug intercalated in LDH

Drug release kinetics were evaluated using the following models:

First order: $\frac{Q_t}{Q_\infty} = 1 - e^{-k_1 \times t}$

Korsmeyer-Peppas (K-P): $\frac{Q_t}{Q_\infty} = k_{K-P} \times t^n$

Higuchi: $\frac{Q_t}{Q_\infty} = k_H \times t^{0.5}$

Parabolic diffusion: $\frac{Q_t / Q_\infty}{t} = k_P \times t^{-0.5} + b$

where, Q_∞ : amount of the drug in the formulation
Q_t : amount of the drug released at the time t
t : release time
k_1 : first-order rate constant
k_{K-P} : Korsmeyer-Peppas kinetic constant
K_H : Higuchi kinetic constant
K_P : Parabolic diffusion constant

2.5. Antibacterial activity and cytotoxicity tests

2.5.1. Antibacterial activity test

Antibacterial effects were investigated using agar disk diffusion, minimum inhibitory concentration (MIC) determined by the resazurin redox indicator, and turbidity measurement.

2.5.2. Cytotoxicity tests

Cytotoxicity was examined for ZnAl-LDH and drug-loaded composites (LEV-LDH, CIP-LDH, SAL-LDH, IBU-LDH) against RAW 264.7 macrophages supplied by ATCC (Manassas, VA, USA) by determining nitric oxide (NO) concentration and performing the MTT assay [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide].

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Characteristics of ZnAl-LDH

- ***Compositional Analyses***

Based on the elemental composition results, the proposed chemical formula of the ZnAl-LDH material is

$$Zn_{1.51}Al_{0.52}(OH)_4(NO_3)_{0.58}\cdot 1.31H_2O$$

- ***TGA, XRD and FTIR***

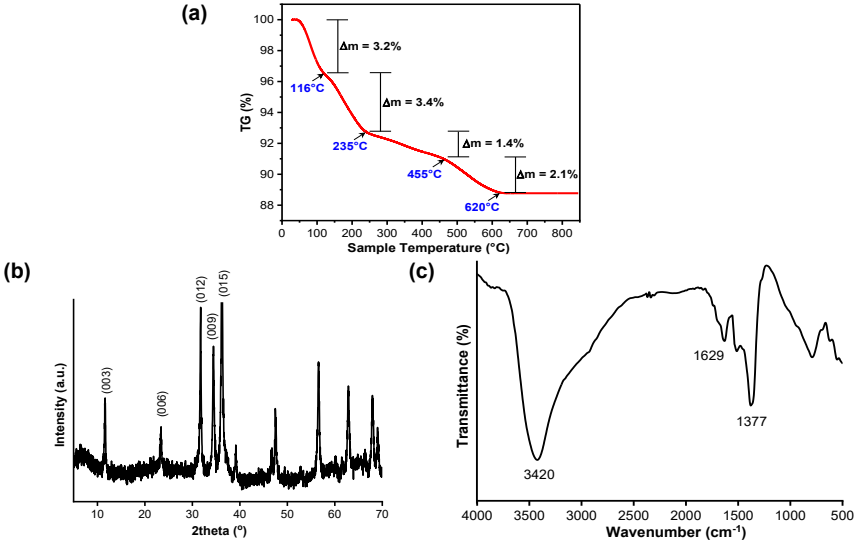


Figure 3.1. *(a) Thermogram; (b) XRD pattern and (c) FTIR spectrum of ZnAl-LDH*

- ***SEM, EDX, DLS***

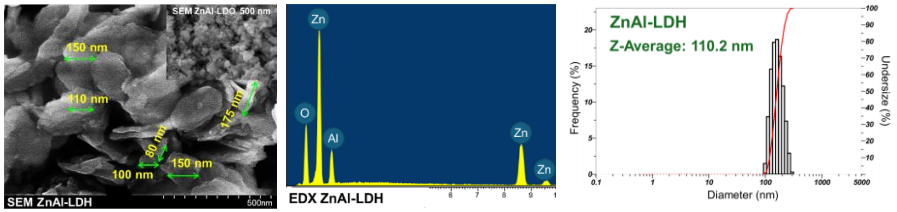


Figure 3.2. *SEM photographs, EDX spectra and the particle size distributions of ZnAl-LDH*

Results confirmed successful ZnAl-LDH synthesis with size of about 110.2 nm.

- ***Antibacterial activity and cytotoxicity of ZnAl-LDH***

ZnAl-LDH exhibited a minimum inhibitory concentration (MIC) of 150 µg/mL against *E. coli* and *S. aureus*. Cell viability of RAW 264.7 macrophages exposed to ZnAl-LDH (10–100 µg/mL) exceeded 80%, suggesting low cytotoxic effects.

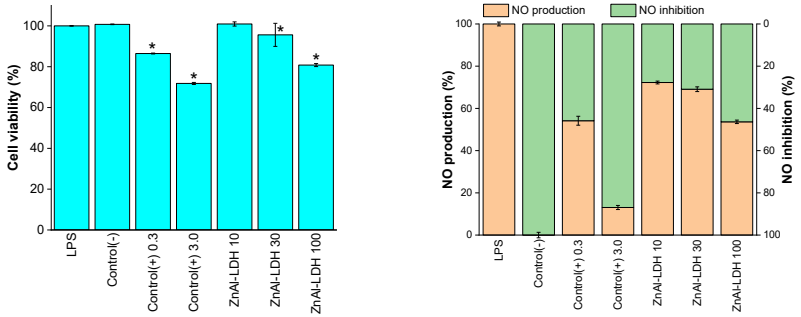


Figure 3.3. *(a) The result of MTT assay and (b) inhibition of NO released by RAW 264.7 of ZnAl-LDH*

3.2. Optimization of drug intercalated into ZnAl-LDH

3.2.1. Optimization of LEV intercalated into ZnAl-LDH

The quadratic equation representing the loading efficiency of LEV can be expressed as:

$$Y_{LEV\text{-}LDH} = 2.25X_1 + 1.40X_2 + 1.89X_3 - 3.06X_1^2 - 1.30X_2^2 - 1.27X_3^2 + 1.20X_1X_3 + 34.79$$

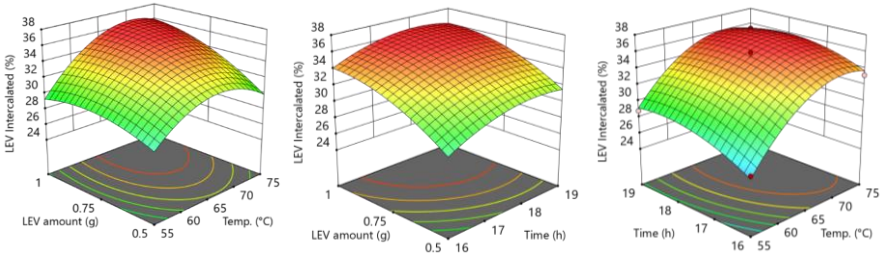


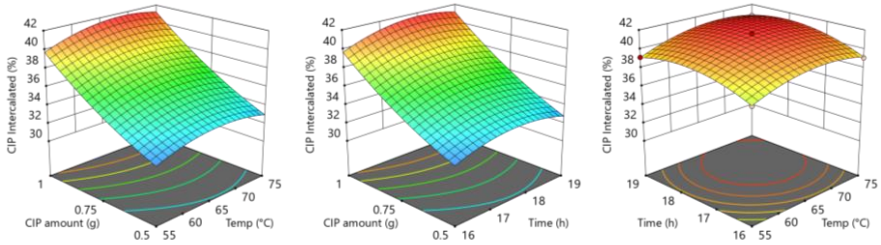
Figure 3.4. *Response surface plots of LEV intercalated into ZnAl-LDH structure at optimum condition.*

Under the studied conditions, optimal LEV loading efficiency was obtained at 70 °C for 18 h with 1.0 g of LEV.

3.2.2. Optimization of CIP intercalated into ZnAl-LDH

The quadratic equation representing the loading efficiency of CIP can be expressed as:

$$Y_{CIP\text{-}LDH} = 0.53X_1 + 0.44X_2 + 3.87X_3 - 1.05X_1^2 - 1.10X_2^2 + 0.66X_3^2 + 0.12X_1X_2 + 0.17X_1X_3 + 0.22X_2X_3 + 36.90$$



(a) (b) (c)

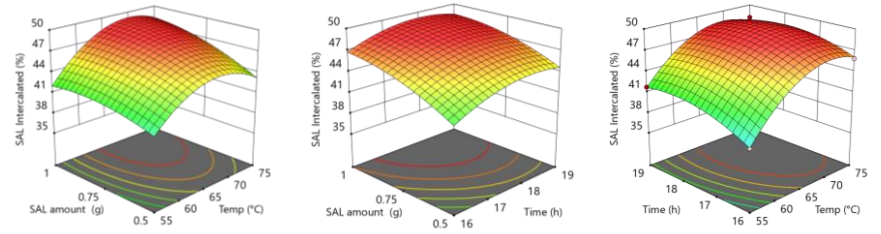
Figure 3.5. *Response surface plots of CIP intercalated into ZnAl-LDH structure at optimum condition*

Under the studied conditions, optimal CIP loading efficiency was obtained at 70 °C for 18 h with 1.0 g of CIP.

3.2.3. Optimization of SAL intercalated into ZnAl-LDH

The quadratic equation representing the loading efficiency of SAL can be expressed as:

$$Y_{SAL\text{-}LDH} = 2.40X_1 + 1.32X_2 + 1.76X_3 - 3.31X_1^2 - 1.42X_2^2 + 0.85X_1X_3 + 47.42$$



(a) (b) (c)

Figure 3.6. *Response surface plots of SAL intercalated into ZnAl-LDH structure at optimum condition*

Under the studied conditions, optimal SAL loading efficiency was obtained at 70 °C for 18 h with 1.0 g of SAL.

3.2.4. Optimization of IBU intercalated into ZnAl-LDH

The quadratic equation representing the loading efficiency of IBU can be expressed as:

$$Y_{IBU\text{-}LDH} = 1.59X_1 + 0.85X_2 + 1.76X_3 - 3.69X_1^2 - 1.04X_2^2 - 0.90X_3^2 + 0.44X_1X_2 + 0.68X_1X_3 - 0.41X_2X_3 + 26.24$$

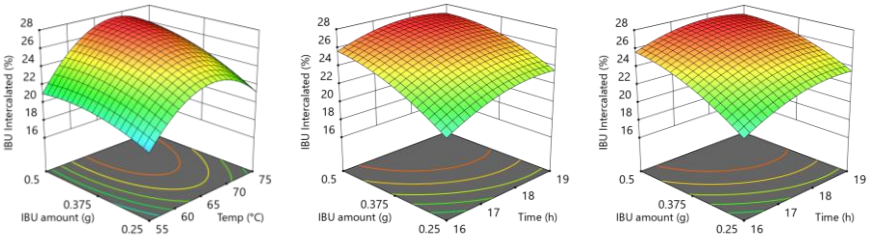


Figure 3.7. *Response surface plots of IBU intercalated into ZnAl-LDH structure at optimum condition*

Under the studied conditions, optimal IBU loading efficiency was obtained at 70 °C for 18 h with 0.5 g of IBU.

3.2.5. Validation of experimental design

Table 3.1. *Drugs intercalated into ZnAl-LDH (predicted value obtained from quadratic equations using optimized conditions)*

Drug (temperature-time-drug amount)	Predicted value (%)	Observed value (%)	Relative error (%)
LEV (70°C-18h-1.0g)	36.56	35.35 ± 1.07	3.31
CIP (70°C-18h-1.0g)	41.63	41.89 ± 1.18	– 0.62
SAL (70°C-18h-1.0g)	49.18	48.00 ± 1.14	2.40
IBU (70°C-18h-0.5g)	27.42	27.51 ± 0.68	– 0.33

3.3. Characteristics of the materials

3.3.1. Compositional Analyses

Based on the results of elemental analyses as well as principle of charge balance, the predicted chemical formulas are:

ZnAl-LDH: $Zn_{1.51}Al_{0.52}(OH)_4(NO_3)_{0.58}\cdot 1.31H_2O$

LEV-LDH: $Zn_{1.44}Al_{0.42}(OH)_4(C_{18}H_{20}FN_3O_4)_{0.26}$

CIP-LDH: $Zn_{1.45}Al_{0.5}(OH)_4(C_{17}H_{18}FN_3O_3)_{0.38}$

IBU-LDH: $Zn_{1.44}Al_{0.49}(OH)_4(C_{13}H_{18}O_2)_{0.35}\cdot 0.8H_2O$

SAL-LDH: $Zn_{1.38}Al_{0.42}(OH)_3(C_7H_6O_3)$

3.3.2. Thermal analysis

TGA profiles of ZnAl-LDH, LEV-LDH, CIP-LDH, SAL-LDH and IBU-LDH as shown below reveal weigh losses attributed to the elimination of the interlayer water and further degradation of inorganic layered to produce mixed metal oxides, or degradation of drugs intercalated in DRUG-LDH.

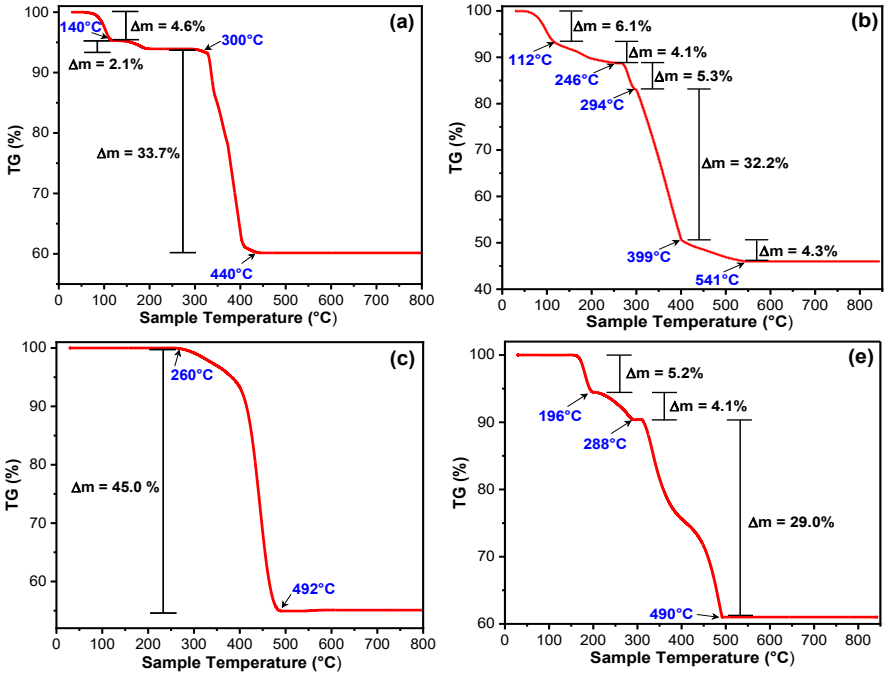


Figure 3.8. *Thermogram for (a) LEV-LDH, (b) CIP-LDH, (c) SAL-LDH và (d) IBU-LDH*

3.3.3. X-ray Diffraction

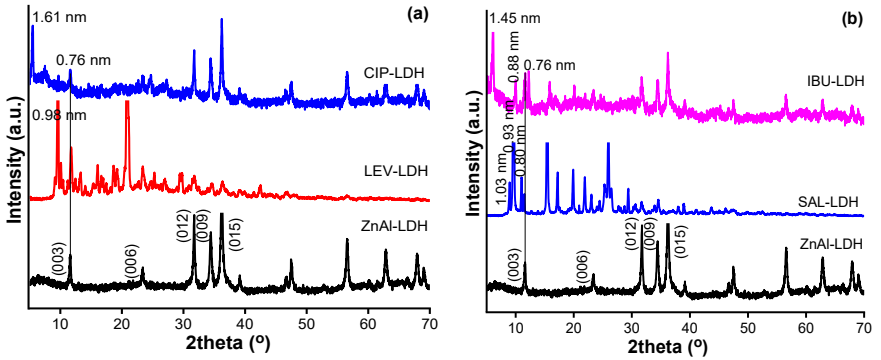


Figure 3.9. *XRD patterns of (a) ZnAl-LDH, LEV-LDH, CIP-LDH and (b) ZnAl-LDH, SAL-LDH, IBU-LDH*

The X-ray diffraction (XRD) profiles of LEV-LDH, CIP-LDH, SAL-LDH, and IBU-LDH showed characteristic peaks consistent with those of ZnAl-LDH, verifying the preservation of the layered structure.

3.3.4. FTIR

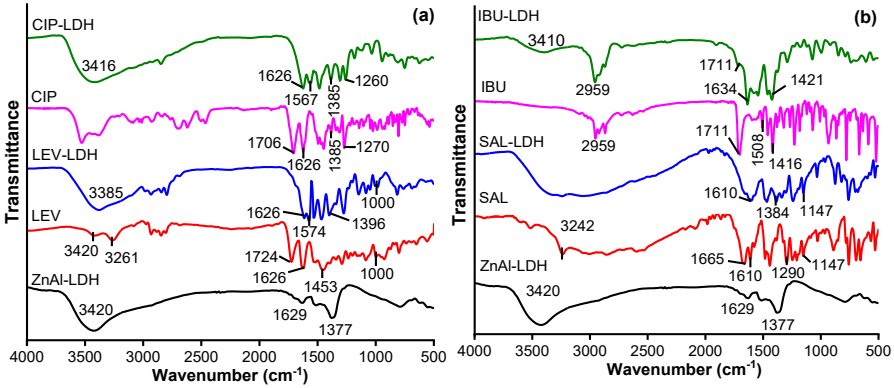


Figure 3.10. *FTIR of ZnAl-LDH, (a) antibiotic drugs (LEV, CIP), LEV-LDH, CIP-LDH; (b) anti-inflammatory drugs (SAL, IBU), SAL-LDH, IBU-LDH*

FTIR analysis shows that the characteristic absorption peaks of the drugs are shifted in LEV-LDH, CIP-LDH, SAL-LDH, and IBU-LDH; indicating chemical interactions between drug functional groups and metal ions located in the ZnAl-LDH structure.

3.3.5. SEM, EDX, DLS

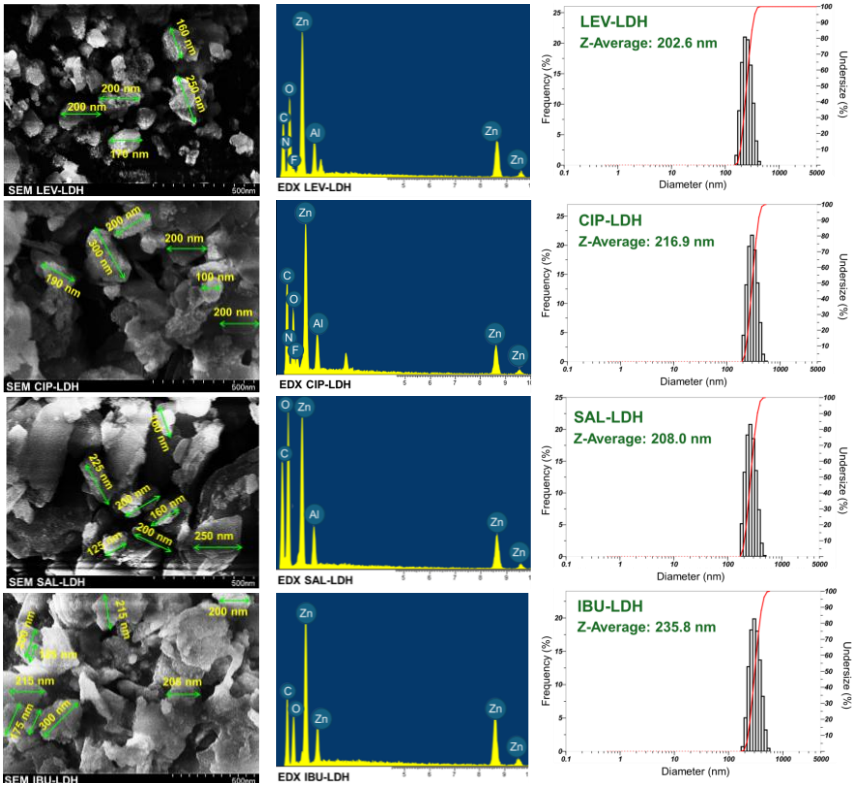


Figure 3.11. *SEM photographs, EDX spectra and the particle size distributions of LEV-LDH, CIP-LDH, SAL-LDH và IBU-LDH*

SEM micrographs of LEV-LDH, CIP-LDH, SAL-LDH, and IBU-LDH revealed that the incorporation of drug anions (LEV, CIP, SAL, and IBU) into ZnAl-LDH led to the formation of stacked plate-like structures. EDX analysis indicated the presence of Zn, Al, C, O, N, and F in LEV-LDH and CIP-LDH, whereas SAL-LDH and IBU-LDH contained Zn, Al, C, and O. The presence of C, O, N, F confirmed drug intercalation into ZnAl-LDH. DLS revealed larger particle sizes for drug–LDH samples: LEV-LDH (~202.6 nm), CIP-LDH (~216.9 nm), SAL-LDH (~208.0 nm), IBU-LDH (~235.8 nm), compared to ZnAl-LDH.

3.4. Drug release study

3.4.1. Drug release properties

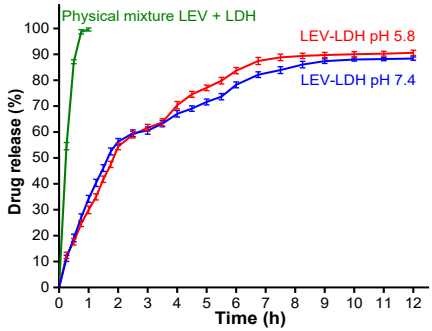


Figure 3.12. *Release profile of LEV from LEV-LDH in PBS (pH 5.8 và pH 7.4)*

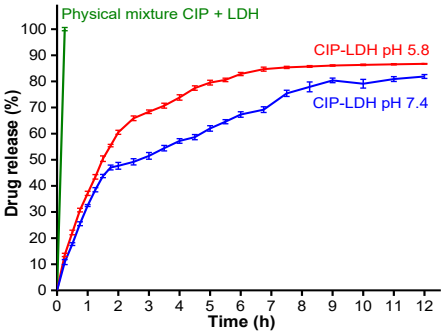


Figure 3.13. *Release profile of CIP from CIP-LDH in PBS (pH 5.8 và pH 7.4)*

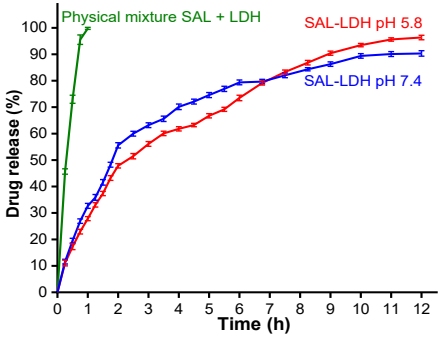


Figure 3.14. *Release profile of SAL from SAL-LDH in PBS (pH 5.8 và pH 7.4)*

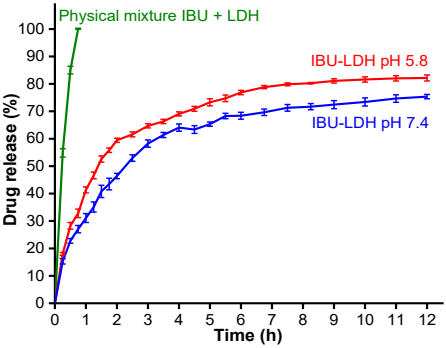


Figure 3.15. *Release profile of IBU from IBU-LDH in PBS (pH 5.8 và pH 7.4)*

Physical mixtures of ZnAl-LDH with drugs (LEV, CIP, SAL, IBU) dissolved completely within 1 h in PBS. LEV-LDH, CIP-LDH, SAL-LDH, and IBU-LDH showed sustained 12 h release, higher at pH 5.8 than at pH 7.4.

3.4.2. Drug release kinetic

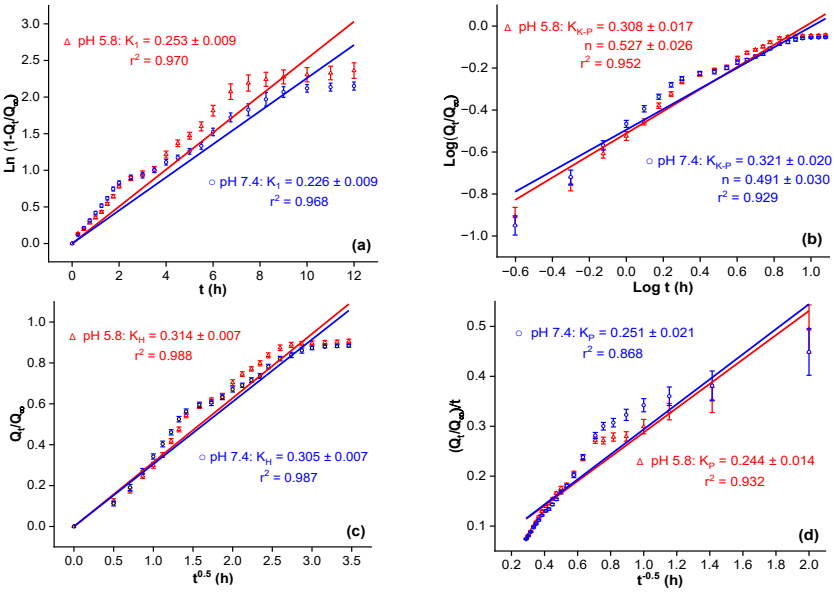


Figure 3.16. *Release kinetics of LEV from LEV-LDH (a) first order, (b) Korsmeyer-Peppas (K-P), (c) Higuchi and (f) parabolic diffusion.*

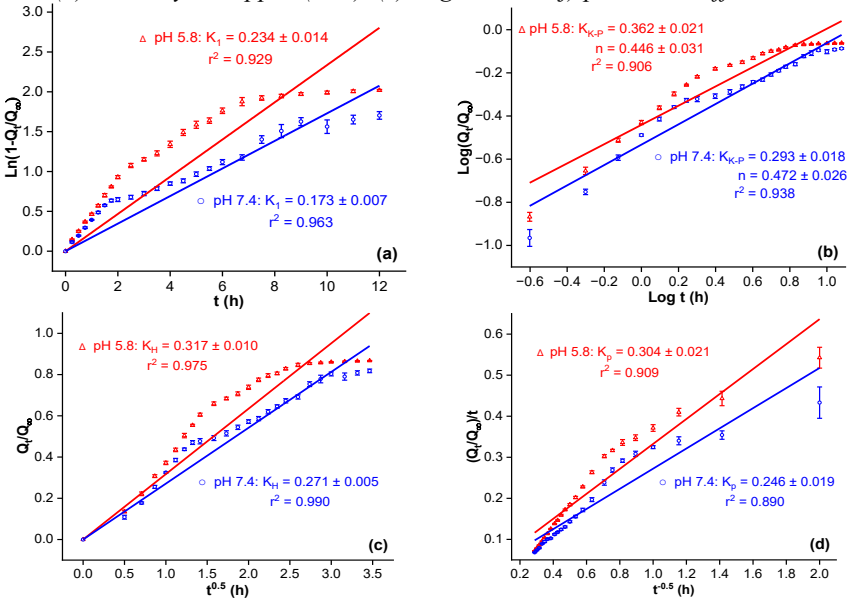


Figure 3.17. *Release kinetics of CIP from CIP-LDH (a) first order, (b) Korsmeyer-Peppas (K-P), (c) Higuchi and (f) parabolic diffusion.*

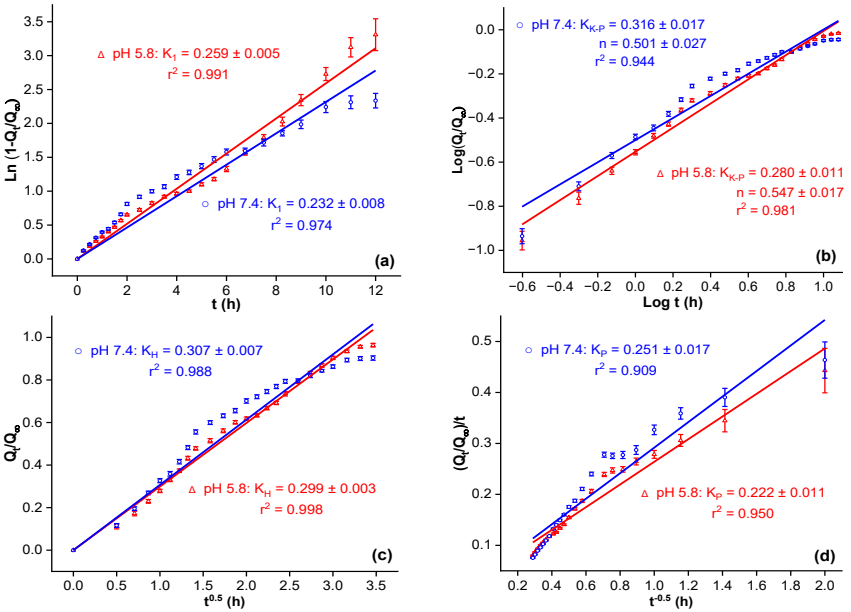


Figure 3.18. *Release kinetics of SAL from SAL-LDH (a) first order, (b) Korsmeyer-Peppas (K-P), (c) Higuchi and (f) parabolic diffusion.*

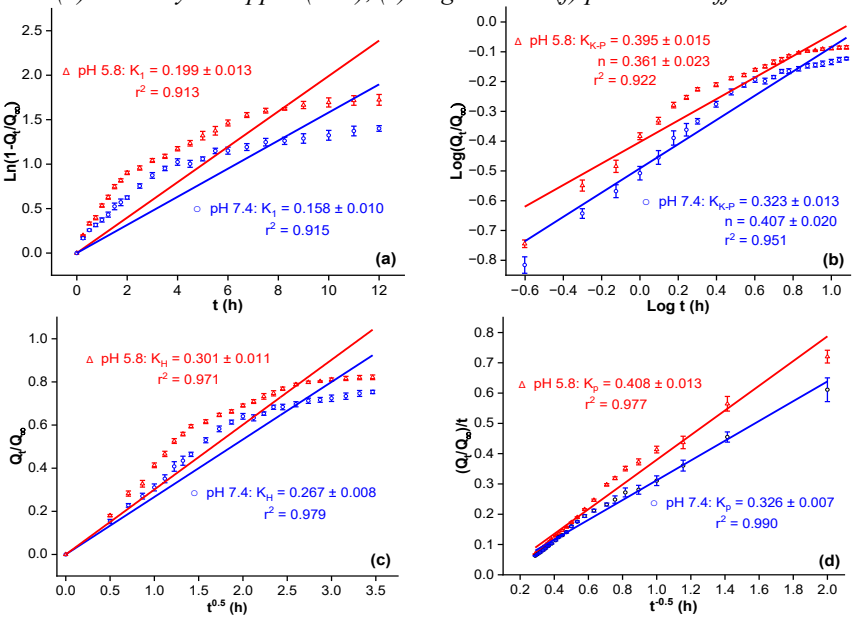


Figure 3.19. *Release kinetics of IBU from IBU-LDH (a) first order, (b) Korsmeyer-Peppas (K-P), (c) Higuchi and (f) parabolic diffusion.*

Drug release is controlled by diffusion, anion exchange, and partial dissolution of ZnAl-LDH.

3.5. Antimicrobial effectiveness and cytotoxicity

3.5.1. Antimicrobial effectiveness

Table 3.2. *Inhibition of ZnAl-LDH, LEV-LDH, CIP-LDH for E.coli and S.aureus*

Materials	Inhibition zone (mm)	
	E.coli	***S.aureus***
Negative control	0	0
Positive control LEV (200 µg/mL)	26.33 ± 0.58	22.00 ± 1.00
Positive control CIP (200 µg/mL)	29.33 ± 0.58	17.67 ± 0.58
ZnAl-LDH (400 µg/mL)	10.33 ± 0.58	8.33 ± 0.58
LEV-LDH (400 µg/mL)	30.67 ± 0.58	25.67 ± 1.15
CIP-LDH (400 µg/mL)	28.33 ± 0.58	19.33 ± 0.58

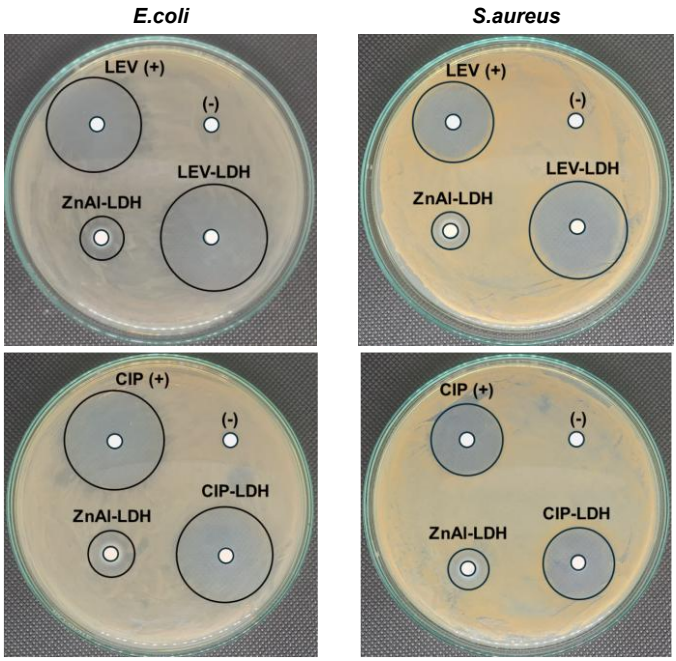


Figure 3.20. *Inhibition zone of ZnAl-LDH, LEV-LDH, CIP-LDH for E.coli and S.aureus*

LEV-LDH and CIP-LDH exhibited markedly improved antibacterial performance compared to pure ZnAl-LDH, likely due to the synergistic interaction between the incorporated drugs (LEV, CIP) and the ZnAl-LDH structure.

3.5.2. Cytotoxicity

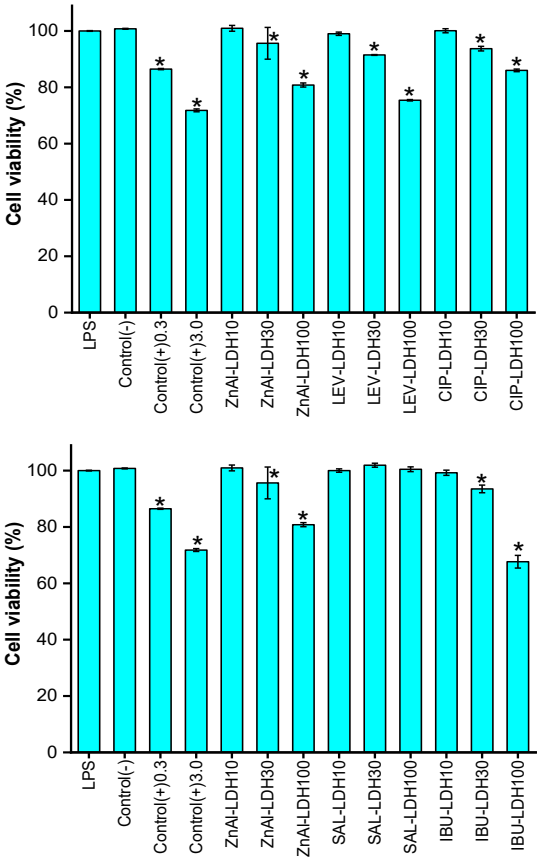


Figure 3.21. *Cell viability of RAW 264.7 (MTT assay)*

Cell viability (%) of RAW 264.7 (at DRUG-LDH 100-10 μg/mL):

- LEV-LDH: from 75.40 ± 0.26 to 99.01 ± 0.58
- CIP-LDH: from 85.99 ± 0.46 to 100.09 ± 0.72
- SAL-LDH: from 100.01 ± 0.61 to 100.47 ± 0.84
- IBU-LDH: from 67.67 ± 2.25 to 99.23 ± 0.93

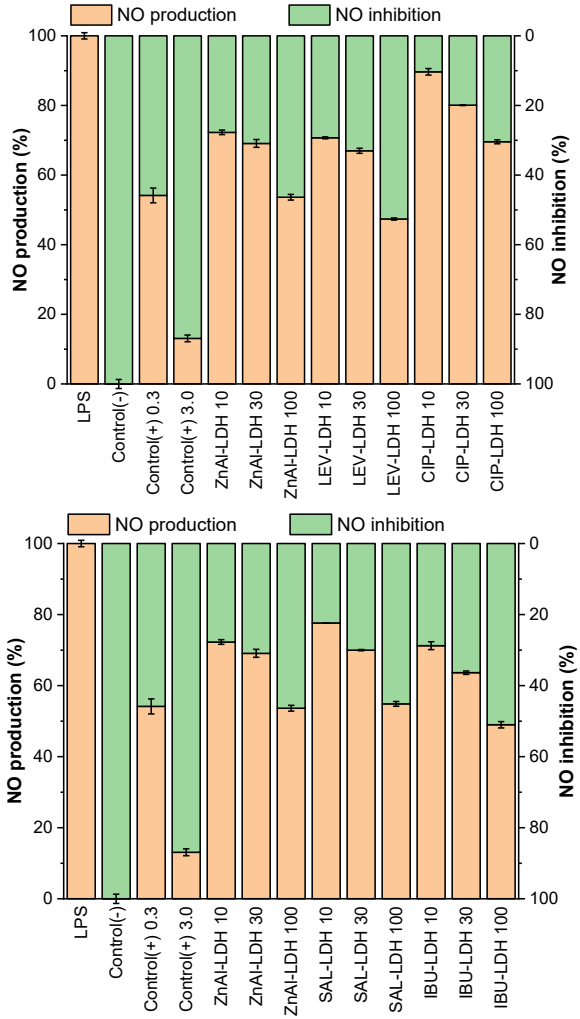


Figure 3.22. *NO inhibition of ZnAl-LDH, LEV-LDH, CIP-LDH, SAL-LDH and IBU-LDH*

NO inhibition (%) (at DRUG-LDH 100-10 μg/mL):

- LEV-LDH: from 22.93 ± 0.28 to 52.62 ± 0.31
- CIP-LDH: from 10.34 ± 0.93 to 30.44 ± 0.56
- SAL-LDH: from 22.38 ± 0.03 to 45.15 ± 0.67
- IBU-LDH: from 28.77 ± 1.11 to 51.02 ± 0.89

CONCLUSION AND RECOMMENDATIONS

Conclusion

In summary, this study on drug-loaded ZnAl-LDH nanomaterials for antibacterial wound healing led to the following findings:

1. ZnAl-LDH nanoparticles (~110 nm) were successfully synthesized using a co-precipitation–ultrasonic method. The material exhibited antibacterial effects against E. coli and S. aureus (MIC = 150 μg/mL) and maintained >80% viability in RAW 264.7 cells (10–100 μg/mL), proving its potential as a wound-healing drug carrier.

2. Using RSM-CCD optimization, the optimal conditions for maximum drug loading were identified at 70 °C for 18 h with 1.0 g of LEV/CIP/SAL and 0.5 g of IBU. The obtained materials were LEV-LDH (35.35 ± 1.07%, 202.6 nm), CIP-LDH (41.89 ± 1.18%, 216.9 nm), SAL-LDH (48.00 ± 1.14%, 208.0 nm), and IBU-LDH (27.51 ± 0.68%, 235.8 nm).

3. LEV-LDH, CIP-LDH, SAL-LDH, and IBU-LDH showed 12 h sustained release (PBS pH 7.4/5.8), enabling prolonged local therapy and fewer doses.

4. Drug release followed a diffusion-controlled mechanism involving anion exchange and gradual dissolution of the ZnAl-LDH layers.

5. LEV-LDH and CIP-LDH exhibited MIC values of 100 μg/mL against *E.coli* and *S.aureus*. Cytotoxicity assays showed > 80% RAW 264.7 viability for LEV-LDH, CIP-LDH, and SAL-LDH (10–100 μg/mL) and for IBU-LDH (10–30 μg/mL), slight cytotoxicity at 100 μg/mL. Concerning anti-inflammatory potential, LEV-LDH, CIP-LDH, SAL-LDH, and IBU-LDH all demonstrated inhibitory effects on NO production. In particular, LEV-LDH and IBU-LDH at a concentration of 100 μg/mL showed a relatively high NO inhibition rate of over 50%.

Recommendations

Expand the scope of investigation regarding synthesis conditions to enhance the drug loading efficiency into the ZnAl-LDH structure.

Test activity against more bacteria to broaden applicability.

Investigate bacterial membrane damage induced by reactive oxygen species (ROS).

Conduct *in vivo* evaluations of LEV-LDH, CIP-LDH, SAL-LDH, and IBU-LDH to assess therapeutic performance in wound models, guiding the development of targeted carriers for inflamed tissues, topical formulations, and controlled-release dosage forms.

Investigate drug release in the pH range of 1–3 to facilitate oral application.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Ngo Thi Tuong Vy, Dang Nguyen Nha Khanh, Pham Duy Khanh, Nguyen Tan Phat, Nguyen The Anh, Nguyen Long Nguyen, Thong Ngoc Lan Anh, Nguyen Ngoc Vy, Le Thi Minh Dan, Nguyen Thi Kim Phuong, *Drug-Intercalated Zn–Al-Layered Double Hydroxides as Antibacterial and Anti-inflammatory Delivery Systems for Wound Healing Applications, Journal of Cluster Science,* 34, 2619–2632, 2023.

2. Ngo Thi Tuong Vy, Dang Nguyen Nha Khanh, Le Hai Khoa, Nguyen Tan Phat, Nguyen Quoc Thang, Le Hoai Trung, Nguyen Thi Huynh Nhu, Doan Thi Minh Phuong and Nguyen Thi Kim Phuong, *Experimental Design Modelling and Optimization of the Intercalation of Levofloxacin and Salicylic Acid into the Structure of ZnAl-LDH by Response Surface Method: in vitro Study of Release Kinetics, Antibacterial and Anti-Inflammatory Activities,* Journal of the Brazilian Chemical Society, 35, 5, 1-14, 2024.

3. Ngo Thi Tuong Vy, Dang Nguyen Nha Khanh, Le Ngoc Thuy Trang, Doan Thi Minh Phuong and Nguyen Thi Kim Phuong, *Optimization of Ibuprofen and Ciprofloxacin Loading on Inorganic 2D Layered Materials via Response Surface Methodology (RSM)-Central Composite Design (CCD) and Application for Antibacterial and Anti-Inflammatory,* Tạp chí phân tích Hóa, Lý và Sinh học, 31, 3A/ 2025.