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PREPARATION AND OPTIMIZING OF A PROMISING NANO-CARRIER FOR CANCER TREATMENT

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ABSTRACT

Cancer has emerged as a leading global cause of mortality. The management of cancer continues to provide a significant challenge. Recent advancements in nanotechnology have led to a significant interest in layered double hydroxide (LDH)-based nanosystems. These nanosystems have gained attention due to their favourable characteristics such as biocompatibility, pH-dependent biodegradability, anion exchange capacity, easy surface modification, and high chemical stability. As a result, they hold great promise for cancer therapy. Over the last ten years, researchers have developed LDH-based nanosystems for a range of cancer treatments. We examined the alterations in the crystal structure and morphology caused by hydrothermal treatment of LDH for 24, 48 hours and the non-hydrothermally treated LDH. We have shown that the time of the hydrothermal treatment may effectively regulate the nanostructures of LDHs, employing scanning electron microscopy, Zeta potential and hydrodynamic diameter measurements, and X-ray diffraction. Our investigation revealed that the hydrothermal treatment induced alteration in the structure of the LDHs. During the initial stage, crystallization took place within the first 24 hours of the hydrothermal treatment, resulting in an expansion of the interlayer width and an increase in the hydrodynamic diameter size. After that, the most prominent occurrences were the stacking and planar expansion that took place as the crystallization process neared equilibrium. Consequently, the previously formed layered structure was also arranged in a stacked manner, causing the LDH to extend in a flat direction. Furthermore, the different treated LDHs greatly affected the loading of chemotherapeutic drug, as the time of hydrothermal treatment increase the concentration of the loaded chemotherapeutic drug decrease. Our research indicates that the length of time the hydrothermal treatment is applied is a critical element in the development of the structure of LDHs. This component

can be utilized to manipulate the structure of LDHs for optimal application as drug carrier.

Key Words: Nano-carrier, hydrothermal, layered structure, cancer treatment.

INTRODUCTION

Cancer is a highly perilous global disease, **(Fan *et al.*, 2017 and Gao *et al.*,2019)**. Presently, the efficacy of clinical cancer treatments remains unsatisfactory due to the absence of appropriate methodologies. Hence, the global community is deeply concerned about the development of innovative approaches to achieve exceptionally effective cancer treatment. A multitude of therapies, including as chemotherapy, phototherapy, gene therapy, immunotherapy, and others, have arisen just at the opportune time, (**Cheng *et al.*, 2014 ; Zhang *et al.*, 2019 and Sahu *et al.*,2020)**. These techniques rely heavily on nanomaterials that possess desired and precise structures, compositions, morphology, and functions. Dimensional nanosheets are a commonly found type of nanomaterials that possess distinct characteristics, including an enormous surface-area-to-mass ratio and certain physicochemical properties. These traits arise from their two-dimensional morphology and extremely thin thickness, **(Tan and Zhang 2015)**. These qualities make them suitable for application as useful compounds in this area of cancer therapies.

Layered double hydroxides (LDHs) consist of a mixture of M^{+2} and M^{+3} hydroxides. These hydroxides form positively charged layers that resemble brucite, with anions located in between the layers. LDHs have garnered considerable interest because of their ability to exchange anions and cations, their aptitude for adsorption, and their prospective applications in medication administration and catalysis. The characteristics of LDHs are intimately linked to their architecture, **(Wen *et al.*, 2021)**.

Intercalating inorganic, organic, or bio molecules into the lamellar lattice of layered double hydroxide (LDH) has posed a significant challenge in the development of new hybrid materials. This process often results in the emergence of dual functionalities or novel mutative features. In recent times, nano-bio convergence technology has emerged as a highly researched area, with a focus on generating sophisticated medications and diagnostic agents to combat diseases and ultimately enhance human well-being, **(Shirin *et al.*, 2021).**

Generally, LDHs with the general formula of $[M^{2+}\ 1–x\ M^{3+}\ x\ (OH)_2]^{X+}\ (A^{n–})_{x/n}.\ mH_2O$ belong to fascinating 2D nanocarriers, in which M^{2+} and M^{3+} are the hydroxides of divalent (e.g., Co^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Ti^{2+}, Cd^{2+}, Ca^{2+}, and Ni^{2+}) and trivalent metal cations (e.g., Al^{3+}, Fe^{3+}, Mn^{3+}, Ga^{3+}, Y^{3+}, In^{3+}, V^{3+}, La^{3+}, Ru^{3+} and Cr^{3+}), respectively,

(Manohara *et al.,* 2021). These anions serve as exchangeable interlayer compensators, neutralizing the positive charges of LDHs interlamellar. Furthermore, the variable n represents the quantity of co-intercalated water molecules that aid in the formation of conjugated multi-layers through hydrogen bonding. The variable x, on the other hand, is associated with the ratio of M3+ ions to the sum of M2+ and M3+ ions, **Yan *et al.,* (2016 & 2017)**. Hydrogen bonding is crucial for maintaining the cohesion of cations and anions, resulting in notable pliability and decreased susceptibility to shear force. The composition of positively-charged metal cations, which stay in the location of the o of the hydroxide layer, necessitates the intercalation of anions to counteract the positive charge of layered structures in a homogeneous profile, **(Mallakpour and Hatami 2020 ; Gholami *et al.,* 2020)**.

Multiple compositional and structural diversities potentially lead to manufacturing distinct forms of LDHs with new intercalates. Recently, various forms of LDHs with fascinating topologies have been designed to efficiently transport diverse medicines to a tumor site, **(Asiabi *et al.,* 2019)**. LDHs with a positive charge can effectively enter cancer cells, depending on the protonation or deprotonation of hydroxyl groups around metal anions. This allows for the controlled release of anticancer drugs, enabling targeted administration of therapies, **(Hakeem *et al.,* 2018 and Yang *et al.,* 2021)**.

Owing to their unique structural features, including the unstable chemical composition, good surface area, and interlayer space, LDHs enable robust adsorption of different hydrophobic/hydrophilic compounds of interest, **(Mir *et al.,* 2021 and Izbudak *et al.,* 2021)**. The impressive characteristics of LDH-based platforms have resulted in significant advancements in their manufacture, leading to efficient therapeutic effects and precise control over the release of contents in a stimuli-responsive manner, **(Wei *et al.,* 2015)**.

In order to achieve specific applications, it is necessary to have a comprehensive comprehension and mastery of the LDH structure for the purpose of predicting and customizing LDH features. The LDH's physical and chemical properties are influenced by its structure, which causes changes in charge density and distribution. Several researches have been conducted to determine the links between the structure and properties of LDHs, **(Zhang *et al.,* 2014 ; Hibino 2015 ; Long *et al.,* 2017 and Yang *et al.,* (2020).**

The present research was aimed to investigate the effect of the hydrothermal treatment in the development of the structure of LDHs and the loading of chemotherapeutic drug, so determining the optimal condition for application of LDH as drug carrier for cancer treatment.

MATERIALS AND METHODS

Materials

All chemicals used in this work were of analytical grade.

METHODS

Preparation of LDH nanostructure

LDH was prepared by co-precipitation method of divalent and trivalent metal salts to form LDH (L) then LDHs were hydrothermally treated (ht) at 140^{o}C for 24 hours (LH1) and for 48 hours (LH2) in a 30 mL hydrothermal autoclave reactor.

Characterizations techniques

Morphological and size analysis

Field emission scanning electron microscope was used for imaging of the powdered samples using accelerating voltage up to 20kV.

X-Ray Diffraction (XRD)

X-ray diffraction was used to document the XRD data. The device worked with a 30 mA current, a functioning voltage of 40 kV (power of 1200 W), and a scanning speed of 2/min (step size = 0.04 degree and step time = 1.0s) in the Start Position [°2Th.] 5.02 End Position [°2Th.] 79.98, 5-80 scanning range (2 Theta scale) at room temperature

Zeta potential and hydrodynamic particle size measurement

The mean hydrodynamic particle size measured by dynamic light scattering (DLS) and the zeta potential of prepared LDHs in different conditions were measured using Nano-Zetasizer, Malvern. For all zeta potential and size measurement suspension was diluted in water.

Chemotherapeutic drug loading measurements

To determine the efficiency of differently treated LDHs to load a chemotherapeutic drug and to test the effect of hydrothermal treatment on the loading of a chemotherapeutic drug. After the preparation of LDHs, a pure chemotherapeutic drug was loaded and the loaded concentration was measured using UV spectrophotometer.

RESULTS AND DISCUSSION

SEM analysis of Figure 1 demonstrates the changes in LDH morphology across various durations of (HT). During the early stages of HT (pristine and 2 h), there were minor observable changes in morphology. Nevertheless, following a 24-hour period of ht, the distinct brucite hexagonal platelet-like architecture, which is a defining feature of hydrotalcites, became apparent. Additional ht resulted in an increase in platelet proliferation, which aligns with earlier findings, **(Ahmed *et al.*, 2012)**.The brucite hexagonal plate-shaped formations became more apparent under scanning electron microscopy (SEM) after 24 hours of ht. It was found a relationship between the size of brucite platelet formations and their duration

under ht conditions. For instance, the brucite hexagonal plate-shaped structure for the LDH (LH1) sample and this size grows as the ht duration increases. After duration of 48 hours, it was seen that the LDHs were largely composed of aggregates of brucite plate-shaped structures.

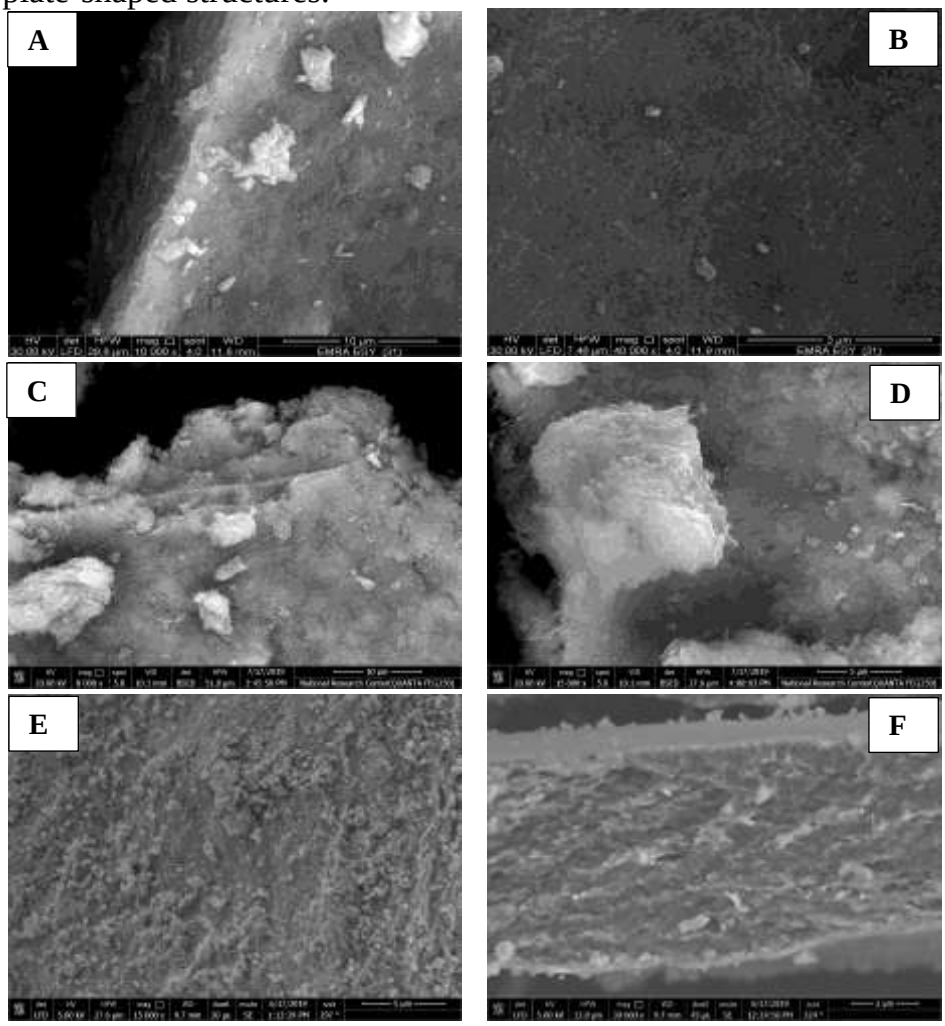

Figure 1: SEM micrographs of the LDH samples of different treatments. A, B: represent L. C, D: represent LH1.E, F: represent LH2.

XRD analysis yields a distinct pattern characterized by well-defined peaks that correlate to the spacing between layers and the crystallographic planes of the hydroxide layers for LDH after 24 hrs without ht. While the hydrothermally treated layered double

hydroxides (LH1 and LH2): The XRD pattern showed alterations in the distance between layers, degree of crystallinity, and composition of different phases resulting from the influence of hydrothermal conditions. XRD is crucial for comprehending the impact of hydrothermal treatment on the structural characteristics of layered double hydroxides (LDHs), aiding in the clarification of alterations in the material's layer arrangement and composition (Figure 2). After 24 hours, there was an enhancement in the atomic arrangement of LDH after high temperature treatment. The enhancement of the atomic arrangement was detected and confirmed using XRD and SEM investigations. The initial qualitative data was acquired through the analysis of the XRD patterns' diffraction lines. These lines grew more distinct, indicating that LDH undergoes crystallization as the duration of HT increases is in agreement with previous study, **(Maegawa *et al.*, 2023).**

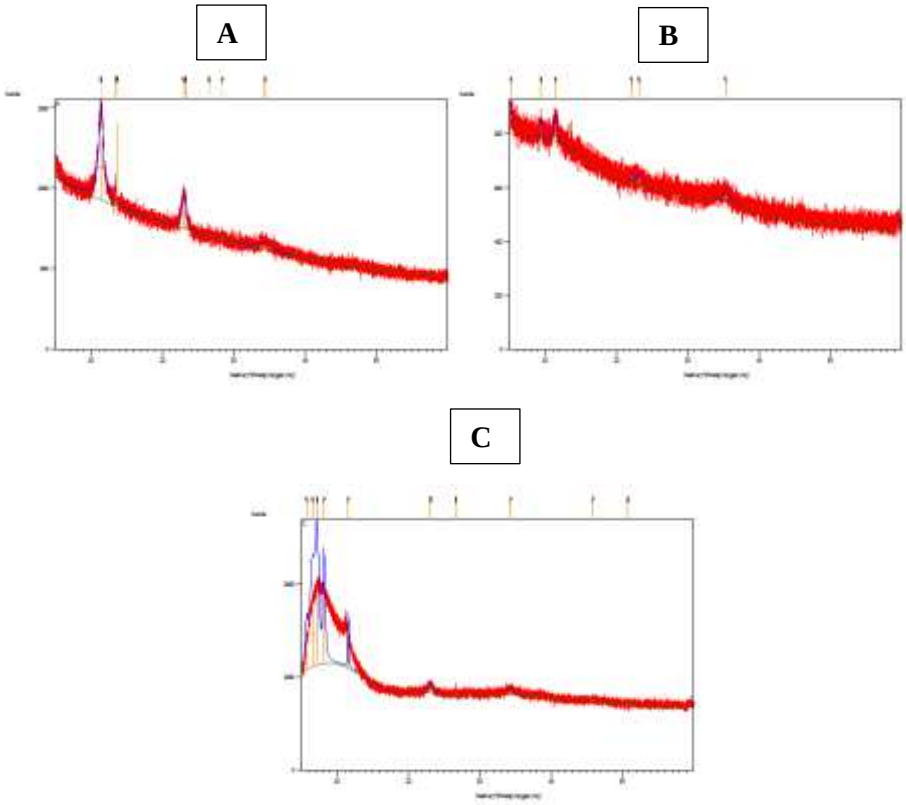

Figure 2: XRD charts of the LDH samples of different treatments. A: represent L. B: represent LH1. D: represent LH2.

The Zeta potential and average hydrodynamic diameter of the prepared LDHs were present in Table 1, which indicated clearly showed the effect of ht and its duration on the zeta potential as well as the hydrodynamic size. The zeta potential of LDH decreased as the time of the ht increases, so it affected on the stability and dispersibility of LDH. The hydrodynamic size of LDH increased with the increase in the time of the ht, which agrees with the SEM micrographs.

Table (1): Zeta potential and hydrodynamic diameter measurements

Sample	Average zeta potential (mV)	Mean hydrodynamic diameter (nm)
L	**10**	**900**
LH1	**8**	**980**
LH2	**6**	**1064**

The loading of chemotherapeutic drug affected by the hydrothermal treatment (ht), as it depressed the loading efficiency of LDH. This might refereed to the effect of ht on the structural layers of LDH and leaded to their compression which affected the loading of bulky chemotherapeutic drug (Table 2).

Table (2): Drug loading percentage

Sample	Drug loading %
L	**11 %**
LH1	**7 %**
LH2	**3 %**

CONCLUSION

The present research indicates that the length of time the hydrothermal treatment is applied is a critical element in the development of the structure of LDHs. Moreover, the different treated LDHs greatly affected the loading of chemotherapeutic drug, as the time of hydrothermal treatment increase the concentration of the loaded chemotherapeutic drug decrease. This component can be utilized to manipulate the structure of LDHs for optimal application as drug carrier.

REFRENCES

Ahmed, A.A.A. ; Z.A. Talib ; M.Z.B. Hussein and A. Zakaria (2012): Improvement of the crystallinity and photocatalytic property of zinc oxide as calcination product of Zn-Al layered double hydroxide. J. Alloys Compd. 2012, 539, 154−160.

Asiabi, H. ; Y. Yamini ; M. Alipour ; M. Shamsayei and S. Hosseinkhani (2019): Synthesis and characterization of a novel biocompatible pseudo-hexagonal NaCa-layered double metal hydroxides for smart pH-responsive drug release of dacarbazine

and enhanced anticancer activity in malignant melanoma. Mater. Sci. Eng., 97: 96–102.

Cheng, L. ; C. Wang ; L. Feng ; K. Yang and Z. Liu (2014): Functional nanomaterials for phototherapies of cancer, Chin. J. Clin. Oncol., 114: 10869–10939.

Fan, W. ; B. Yung ; P. Huang and X. Chen (2017): Nanotechnology for multimodal synergistic cancer therapy, Chem. Rev., 117: 13566–13638.

Gao, P. ; W. Pan ; N. Li and B. Tang (2019): Boosting cancer therapy with organelle-targeted nanomaterials, ACS Appl. Mater. Interfaces, 11: 26529–26558.

Gholami, P. ; L. Dinpazhoh ; A. Khataee ; A. Hassani and A. Bhatnagar (2020): Facile hydrothermal synthesis of novel Fe-Cu layered double hydroxide/biochar nanocomposite with enhanced sonocatalytic activity for degradation of cefazolin sodium. J. Hazard Mater., 381:120742.

Hakeem, A. ; G. Zhan ; Q. Xu ; T. Yong ; X.Yang and L. Gan (2018): Facile synthesis of pH- responsive doxorubicin-loaded layered double hydroxide for efficient cancer therapy. J. Mater. Chem.;6:5768–74.

Hibino, T. (2015): Layered double hydroxide-agarose composites for water treatment: Carbonate contamination during the drying process. Appl. Clay Sci., 116−117: 93−101.

Izbudak, B. ; B. Cecen ; I. Anaya ; K. Miri; A. Bal-Ozturk and E. Karaoz (2021): Layered double hydroxide-based nanocomposite scaffolds in tissue engineering applications. RSC Adv.,11: 30237–52.

Long, J. ; Z. Yang ; J. Huang and X. Zeng (2017): Self-assembly of exfoliated layered double hydroxide and graphene nanosheets for electrochemical energy storage in zinc/nickel secondary batteries. J. Power Sources, 359: 111−118.

Maegawa, K. ; F. Zhang ; Q. Johnson ; M. Jitianu ; W.K. Tan ; G. Kawamura ; A. Matsuda and A. Jitianu (2023): Control of Micro- and Nanostructures of Layered Double Hydroxides by Hydrothermal Treatment. Crystal Growth & Design., 23 (4): 2128-2137.

Mallakpour, S. and M. Hatami (2020): Highly capable and cost-effective chitosan nanocomposite films containing folic acid-functionalized layered double hydroxide and their *in vitro* bioactivity performance. Mater. Chem. Phys., 250: 123044.

Mir, Z.M. ; C. Gomes ; A.C. Bastos ; R. Sampaio ; F. Maia and C. Rocha (2021): The stability and chloride entrapping capacity of

ZnAl-NO2 LDH in high-alkaline/cementitious environment. Corros Mater. Degrad., 2:78–99.

Sahu, A. ; I. Kwon and G. Tae (2020): Improving cancer therapy through the nanomaterial sassisted alleviation of hypoxia, Biomaterials, 228: 119578.

Shirin, V.A. ; R. Sankar ; A.P. Johnson ; H. Gangadharappa and K. Pramod (2021): Advanced drug delivery applications of layered double hydroxide. J. Controlled Release, 330: 398–426. **Manohara, G. ; D. Norris ; M.M. Maroto-Valer and S. Garcia (2021):** Acetate intercalated Mg–Al layered double hydroxides (LDHs) through modified amide hydrolysis: A new route to synthesize novel mixed metal oxides (MMOs) for CO 2 capture. Dalton Trans., 50:7474–83.

Tan, C. and H. Zhang (2015): Epitaxial growth of hetero-nanostructures based on ultrathin two-dimensional nanosheets, J. Am. Chem. Soc. 137 (2015) 12162–12174.

Wei, P.R. ; Y. Kuthati ; R.K. Kankala and C.H. Lee (2015): Synthesis and characterization of chitosan-coated near-infrared (NIR) layered double hydroxide-indocyanine green nanocomposites for potential applications in photodynamic therapy. Int. J. Mol. Sci.,16:20943–68.

Wen, J. ; K. Yang ; J. Huang and S. Sun (2021): Recent advances in LDH-based nanosystems for cancer therapy. Materials & Design, 198: 109298.

Yan, K. ; G. Wu and W. Jin (2016): Recent advances in the synthesis of layered, double- hydroxide-based materials and their applications in hydrogen and oxygen evolution. Energ. Technol.; 4:354–68.

Yan, L. ; M. Zhou ; X. Zhang ; L. Huang ; W. Chen and V.A.L. Roy (2017): A novel type of aqueous dispersible ultrathin-layered double hydroxide nanosheets for in vivo bioimaging and drug delivery. ACS Appl. Mater Interfaces; 9:34185–93.

Yang, H. ; Z. Chen ; P. Guo ; B. Fei and R. Wu (2020):B-doping-induced amorphization of LDH for large-current-density hydrogen evolution reaction. Appl. Catal. B Environ., 261: 118240.

Yang, Z. ; C. Zhang ; G. Zeng ; X. Tan ; D. Huang and J. Zhou (2021): State-of-the-art progress in the rational design of layered double hydroxide based photocatalysts for photocatalytic and photoelectrochemical H2/O2 production. Coord. Chem. Rev., 446: 214103.

Zhang, P. ; T. Yamaguchi ; B.N. Nair ; K. Miyajima ; G.M. Anilkumar (2014): Mg-Al layered double hydroxides: A

correlation between synthesis-structure and ionic conductivity. RSC Adv., 4: 41051−41058.

Zhang, X. ; G. Li ; D. Wu ; X. Li ; N. Hu and J. Chen (2019): Recent progress in the design fabrication of metal-organic frameworks-based nanozymes and their applications to sensing and cancer therapy, Biosens. Bioelectron., 137: 178–198.

تحضير وتحسين حامل نانوي واعد لعلاج السرطان

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لقد برز السرطان كأحد الأسباب الرئيسية للوفيات على مستوى العالم. ولا يزال إدارة السرطان تمثل تحديًا كبيرًا. وقد أثارت التقدمات الحديثة في تكنولوجيا النانو اهتمامًا كبيرًا في الأنظمة النانوية المستندة إلي مركبات النانو من طباقات مزدوجة الهيدروكسيل (LDH) وقد نالت هذه الأنظمة النانوية اهتمامًا نظرًا لخصائصها الملائمة مثل التوافق البيولوجي، القابلية للتحلل البيولوجي المعتمد على درجة الحموضة، قدرة تبادل الأيونات، سهولة تعديل السطح، والاستقرار الكيميائي العالي. ونتيجة لذلك، فإنها تحمل وعدًا كبيرًا في علاج السرطان. على مدار العشر سنوات الماضية، طور الباحثون أنظمة نانوية قائمة على LDHالمجموعة من علاجات السرطان .في هذا البحث تم دراسة التغيرات في بنية البلورات والمورفولوجيا الناتجة عن المعالجة الهيدروثرمالية لـ LDHعلى مدى 24 و48 ساعة، مقارنةً بـ LDH غير المعالج هيدروثرمياً. وقد أظهرنا أن مدة المعالجة الهيدروثرمالية يمكن أن تنظم بشكل فعال الهياكل النانوية لـ LDH، باستخدام مجهر المسح الإلكتروني، وقياسات الجهد الساكن وقطر الهيدروديناميكي، والتحليل بالأشعة السينية. كشفت تحقيقاتنا أن المعالجة الهيدروثرمالية تسببت في تغيير بنية الـ .LDHخلال المرحلة الأولية، حدثت التبلور خلال أول 24 ساعة من المعالجة الهيدروثرمالية، مما أدى إلى توسع عرض الطبقات وزيادة في حجم القطر الهيدروديناميكي. بعد ذلك، كانت أبرز الظواهر هي التراص والتوسع السطحي الذي حدث مع اقتراب عملية التبلور من التوازن. وبالتالي، تم ترتيب الهيكل الطبقي الذي تم تشكيله سابقًا بطريقة مكدسة، مما أدى إلى تمدد الـ LDHفي الاتجاه المسطح. علاوة على ذلك، أثرت LDHsالمعالجة بشكل كبير على تحميل الأدوية الكيميائية، حيث زاد زمن المعالجة الهيدروثرمالية وانخفضت تركيزات الأدوية الكيميائية المحملة. تشير أبحاثنا إلى أن مدة زمن المعالجة الهيدروثرمالية عنصر حاسم في تطوير بنية الـ .LDH يمكن استخدام هذا العنصر للتلاعب بهيكل الـ LDH لتحقيق أفضل تطبيق كحامل للأدوية.