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Synthesis and characterization of Gabapentin-Zn_2Al-LDH nanohybrid and investigation of its drug release and biocompatibility properties on a laboratory scale

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[1]LDH: Layered Double Hydroxide
[2]MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

ABSTRACT

Due to their high biocompatibility, Layered Double Hydroxides can be used as hosts in drug delivery systems. In Layered Double Hydroxide-drug hybrids, medicinal properties including stability in the physiological environment of the Body are investigated. The current research work is the synthesis and characterization of a new hybrid of LDH[1], which forms the nanocomposite of Gabapentin-Zn_2Al-LDH. Gabapentin was selected as a drug model and intercalated in Zn_2Al-LDH layers by co-precipitation method under a nitrogen atmosphere. To determine the structural characteristics of the Gabapentin-Zn_2Al-LDH nanohybrid, the structure of Gabapentin-Zn_2Al-LDH was investigated under X-ray diffraction, Fourier transform infrared spectroscopy, and scanning electron microscopy. The size of nanohybrid particles in three dimensions X, Y, and Z was reported as 72, 43, and 58 nm, respectively. Finally, to evaluate biocompatibility and drug release properties, drug delivery and MTT[2] assay tests for biocompatibility were performed. and acceptable results were obtained.

Introduction

Recent scientific studies have shown that when drugs are intercalated into layered double hydroxides, also known as anionic clays or hydrotalcite-like materials, it can reduce negative side effects like stomach irritation and enhance the solubility of the drugs. [1]. LDHs offer the notable advantages of being cost-effective, biocompatible, minimally cytotoxic, and providing full protection for loaded drugs while allowing for easy preparation. [2]. The chemical industry and academic researchers are highly intrigued by LDHs, a specific type of anionic clays, for their wide-ranging applications in catalysis, electrode materials, polymer additives, drug delivery systems, and release mechanisms. [3],[4],[5],[6]. LDHs are typically represented by the formula $[M^{2+}_{1-x}M^{3+}_x(OH)_2]\ (A^{n-})_{x/n}\cdot mH_2O$, where M^{2+} and M^{3+} are divalent and trivalent metal cations, correspondingly, and An^- is a functional anion [7]. Positively charged host layers are formed by the partial replacement of M^{2+} with M^{3+}, which balances out with the interlayer anions in a framework consisting of brucite-like layers made up of edge-sharing octahedra ($M(OH)_6$) [8].

Layered double hydroxides are widely considered as a potential solution for delivering chemotherapeutic drugs and bioactive molecules to mammalian cells in the laboratory and in vivo. This drug delivery system has shown promise for many years. This system offers high levels of efficiency and drug loading capacity. It also effectively protects the loaded molecules from degradation. Compared to other commonly used nanoparticles such as iron oxide, silicon dioxide, and single-walled carbon nanotubes, LDH is biocompatible in toxicity studies [9].

LDH nanohybrids can be prepared by simple coprecipitation, anion exchange, and regeneration methods also used in advanced drug delivery and controlled release systems [10], biosensors, and genetically encoded systems, among other lesser-known applications [11,12]. Because LDH can incorporate biomaterials in the interlayer region, adsorb substances with its large surface area, and has a

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flexible structure and swelling properties and high chemical and thermal stability for drug modulation., has become a smart excipient with high technological potential for the pharmaceutical industry, drug release, high biocompatibility and easy synthesis [13]. Del Arco et al. synthesized the fenbufen compound in three different ways, each from magnesium, aluminum, and iron hydrotalcites**,** respectively by three different methods, i.e. co-precipitation, ion exchange of chloride anions in the middle layers and regeneration method by hydrotalcite containing carbonate. According to these authors, fenbufen effectively intercalated in the space of layered double hydroxides [14]. Duan et al. synthesized naproxen in magnesium aluminum hydrotalcite with a nitrate interlayer by an ion exchange method at 70 °C and pH= 8 under a nitrogen atmosphere (to prevent carbonization). Under the experimental conditions during exchange, complete replacement of nitrate by naproxen occurs, but slight carbonate contamination is inevitable. The interlayer space corresponding to the height of the gallery contains 1.86- nanometer naproxen molecules, which include a double naphthalene ring that is perpendicular to the magnesium-aluminum hydrotalcite brucite like layers. And the carboxylate groups alternately located at the upper and bottommost of interlayered layer. And molecules of water are placed between naproxen and quasi-brucite layers [15].

Gabapentin, derived from gamma-aminobutyric acid, was originally developed for the treatment of epilepsy, but has expanded its scope to include multiple uses, including the treatment of nerve pain such as headaches and lower back pain. The molecular formula of gabapentin structure is $C_9H_{17}NO_2$ and the molecular weight is171.24. (Figure 1)

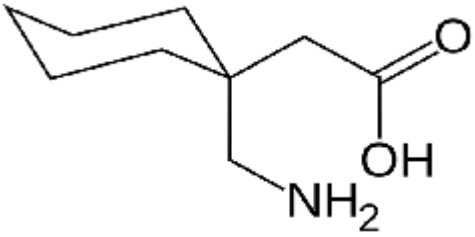

Fig. 1. Molecular structure of gabapentin

A regular review and detailed meta-analysis by Clark et al. In 2012, a final review of eight studies on gabapentin and three studies on pregabalin found that all four studies in the gabapentin group and three studies in the pregabalin group reduced pain frequency and pain medication use**.** It was shown that his six longitudinal studies also examined long-term performance criteria. Four studies found that the use of gabapentin and pregabalin improved long-term functional outcomes in patients. Of course, studies with positive results used higher doses of the drug before surgery and continued the drug regimen after surgery [16]. Another systematic review found that the use of gabapentin before surgery significantly reduced postoperative pain compared to a control group, further reducing the need for opioids and minimizing associated side effects [17].

In some studies, theoretical and experimental work has been performed on the characterization of different intercalated molecules into Zn-Al-LDHs [18]. The works done in recent years by people such as Haraketi et al. have been done on the interaction of the salicylic acid drug with the layered double hydroxides of Zn-Al and Mg-Al for the drug release. Using X-ray diffraction, they displayed that the distance the base of salicylate layered double hydroxide is increased [19] Also, Shen et al. have studied the synthesis and properties of drug release from nanofibers containing layered double hydroxides, the drug beta-naphthoxyacetic acid intercalated Mg-Al-LDHs [20]. Bashi et al. conducted a study on the intercalation of layered double hydroxides nanohybrid with folic acid for use as a delivery system, and their corresponding controlled release properties [21]. Celis et al. investigated the addition of fatty acids to layered double hydroxides [22]. Since no one has done any significant research on the binding of gabapentin and LDH, in this study we synthesized double hydroxide nanoparticles with a zinc-aluminum layer and used it as an inorganic compound to release gabapentin as a nanocarrier. For the synthesis of LDH, the optimal pH for LDH synthesis is 8-9 and metal ratio Zn/Al=2. In this state, the peak intensity is high because the product contains almost no impurities.

2. Experimental

2.1. Material

All chemicals used contain Zn $(NO_3)_2$. $6H_2O$, Al $(NO_3)_3$. $9H_2O$, NaOH, and HNO_3 were purchased from Merck Elman Company. The deionized water and Gabapentin drug were obtained from Tehran Chemical Company.

2.2. The coprecipitation method of preparing layered double hydroxides based on zinc-aluminum.

In this method, we dissolve 0.006 g of gabapentin in 40 cc of deionized water and pass it through a sieve, then we dissolve it inside a three-hole flask containing 40 ml of deionized water under nitrogen gas, then 0.004 mol (1.189 g) Zn $(NO_3)_2$. $6H_2O$ and 0.002 mol of Al $(NO_3)_3$. $9H_2O$ (0.750 g) in 40 ml of deionized water under nitrogen gas and vigorous stirring, then NaOH solution(1N) was added drop by drop to the above solution until the pH of the solution was set at 8. We place the solution for 72 hours at a temperature

of 70°C on a mixer heater, at a constant pH and under reflux. The resulting sediment is washed three times with deionized water and dried for 48 hours.

2.2. Measuring the amount of drug release by spectrophotometer

This step is performed by preparing a solution containing a known concentration of gabapentin as a control solution and reading the absorbance at the maximum wavelength of gabapentin (210 nm). First, to perform this test, dissolve a whole tablet of PBS in 500 ml of distilled water, then dissolve 0.02 g of gabapentin-Zn_2Al-LDH compound in 200 ml of buffer. The resulting solution has a temperature of 37 degrees Celsius**,** a pH of 7.4 bar, a speed of 100**,** and is mixed in about 30 minutes. Then, the absorbance of 5 ml of nanohybrid buffer is measured at wavelength 210 using a spectrophotometer**.** After 10 minutes, measure the second 5 ml of releasable solution after centrifugation. Then, 5 ml of pure new buffer (500 ml) is added to the nanohybrid buffer while stirring and the mixture is allowed to stand for 10 minutes. Continue the above process until the concentration and absorbance show a constant value and continue.

2.3. Investigation of drug release in laboratory scale

The amount of replaced drug is measured by a spectrophotometer that can work in the visible and ultraviolet range at a wavelength of 210 nm. Firstly, to determine the total amount of absorbed drug, 0.02 grams of Gabapentin-Zn_2Al-LDH was dissolved in 6 molars of HCl. By measuring its absorbance(A) at 210 nm, the concentration(c) is achieved from the equation $A = \varepsilon\ b\ c$ [23].So that A is the absorbance, ε is the molar attenuation coefficient ,b is the optical path length and c is the concentration of the attenuating species. To determine ε, the absorption is measured three times for drug concentrations of 0.02, 0.04, and 0.06 molar. If the values of absorbance are placed in the relation $A = \varepsilon\ b\ c$, the values of ε are obtained, and their average is the final value of ε. Then the measured absorptions in the drug release process can be in the relation of $A = \varepsilon\ b\ c$ and the concentrations are obtained in different absorptions. The relation of the release percentage is equal to the ratio of the concentration of the drug (Gabapentin-Zn_2Al-LDH) to the concentration of drug (Gabapentin-Zn_2 Al-LDH) in 6 molars of hydrochloric acid. Finally, the graph of the release percentage is attained according to the time.

2.4. MTT assay

The MTT test is a colorimetric method based on the reduction and breaking of yellow tetrazolium crystals by the enzyme succinate dehydrogenase and the formation of insoluble blue crystals [24]. In this method, unlike other methods, the washing steps, which often cause the loss of a number of the cells are removed and all test steps are performed from the beginning of cell culture to reading the results with a spectrophotometer in a microplate.

3. Results and discussion

3.1. Comparison of FTIR spectrum for Gabapentin drug substance and Gabapentin-Zn_2Al-LDH compound

The Fourier-transform infrared spectroscopy of Gabapentin and Gabapentin-Zn_2Al-LDH drug is shown in Figure 2. In the case of gabapentin in Figure 2 (a), strong absorption is not seen in band 3440cm^{-1} due to the absence of non-acidic OH, and the slight peak around 3100 cm^{-1} is related to OH of the carboxylic acid group. Also peak of 2853cm^{-1} is related to the alkyl groups of the cycloalkyl functional group, which covers a large part of the drug molecule. The peaks of 2602 cm^{-1} and 2250 cm^{-1} are related to pharmaceutical impurities of extract in the project. The peak of 1550 cm-1is related to the remained nitrogenous gases in LDH. Also, the bond of NH of amine group in peak 3400cm^{-1} with OH of acidic group in peak 3100 cm^{-1} is affected.

In the spectrum of Gabapentin Zn_2Al-LDH in Figure 2 (b), a strong broad absorption band with a centrality of 3442 cm^{-1} is related to the stretching vibrations of hydroxyl groups and surface and intralayer water molecules [25]. Compared to free water molecules, the O-H stretching vibrations which appear at 3600 cm^{-1}, this peak appears at a lower frequency in LDHs. This is related to the formation of hydrogen bonds between the interlayer water and various guest anions as well as with the hydroxide groups of the layers. The peak corresponding to 608 related to the C-H bending motions in the structure of gabapentin molecule the bands centered at 590 cm^{-1} and 620 cm^{-1} are related to Zn–O and Al–O lattice vibrations.[26] By comparing the absorption frequencies related to(a) Gabapentin and (b)Gabapentin-Zn_2Al-LDH), there is a relative agreement of the 3100 cm^{-1} peak related to the acidic OH group and peaks of 1630-1690cm^{-1} related to the stretching vibration group of the carbonyl group in the compounds with the first type of amine. Therefore, can be deduced that the above peaks both in the drug and LDHs are preserved, indicating the interlaying of the gabapentin drug in LDHs inter-layer space.

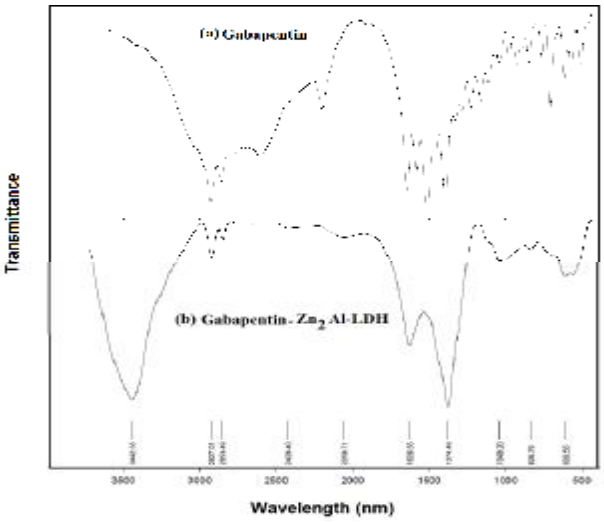

Fig. 2. FT-IR spectrum of (a Gabapentin (b) Gabapentin Zn_2Al-LDH

3.2. X-ray spectroscopic analysis of Gabapentin-Zn_2 Al-LDH (XRD)

Figure. 3(a) the XRD pattern of Zn_2Al-NO_3-LDH [27] and Figure 3(b) the XRD pattern of Gabapentin-Zn_2Al-LDH shows the reflections associated with the crystalline layer phase which in the range of 2 to 70 degrees. A strong and sharp peak for Zn_2Al-NO_3-LDH is observed at lower values of 2Θ, shown in Figure 3, and represents the good crystallinity of Zn_2Al-NO_3-LDH as a standard material. In the Zn_2Al-NO_3-LDH structure, the basic space is d_{003}=8.73Å. This space is the sum of the thickness of the brucite like layers (0.48 nm) and the height of the interlayer space, which is a function of the number, size, and orientation of the placed anions. The XRD pattern of the sample in Figure 3 shows that during the placement of gabapentin molecules, the LDH layers open so that they can accommodate the anions inside. This expansion is seen in the value of 9.87 Å equal to d_{003}. The expansion of the layers due to the expansion of the base space related to the scattering by the d_{003} planes is much larger than that in pure LDH. The thickness of the LDH layers is 4.80 Å, so only 5.07 Å of the 9.87 Å are left as useful interlayer space for placement.

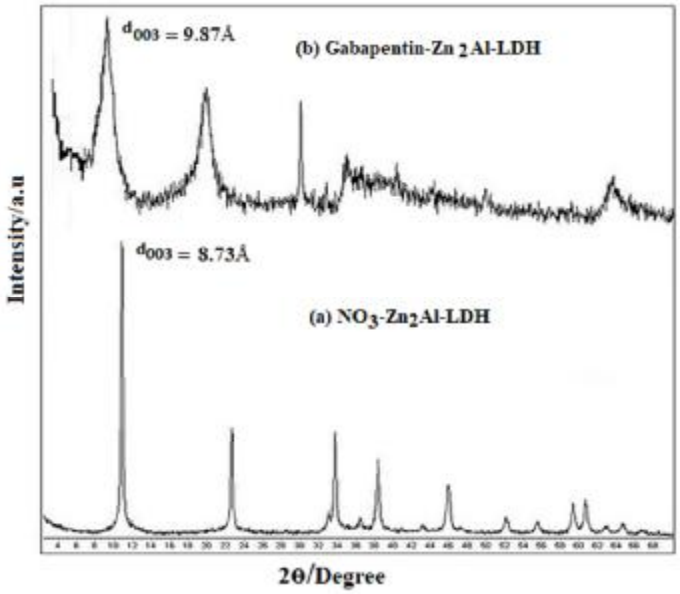

Fig. 3. XRD patterns of the precursor of Zn_2Al-NO_3-LDH(a) and Gabapentin-Zn_2 Al-LDH (b)

3.3. Scanning Electron Microscopy (SEM) analysis for Gabapentin-Zn_2Al-LDH

SEM images of Gabapentin Zn_2Al-LDH nanohybrid with two magnifications at 600 µm and 500 nm are shown in Figure 4. The irregular feature of the pebble-like Gabapentin-Zn_2Al-LDH nanohybrid can be well seen in Figure 4. Gabapentin-Zn_2Al-LDH nanohybrids are in the form of nano-pebbles, which have a small thickness and the size of 58-43-72nm particles in three dimensions X, Y, and Z.

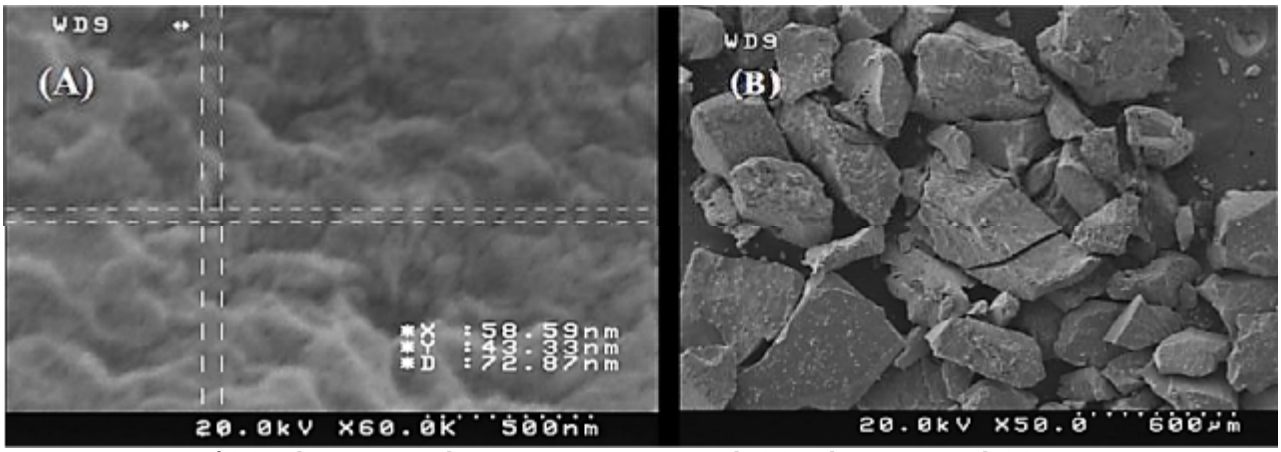

Fig. 4. SEM images of (A) Gabapentin-Zn_2Al-LDH system at 500 nm scale (A) and at 600 µm scale(B)

3.4. Energy-dispersive X-ray (EDX) spectroscopy

The analysis of the chemical composition of the elements related to the substance Gabapentin-Zn_2Al-LDH was performed at the microscopic level by energy-dispersive X-ray spectroscopy (EDX). EDX analysis is shown in Figure 5. The presence of Zn, O, and Al, peaks indicate the presence of the main elements of LDH composition, as well as C, H, N, and O elements, indicating the presence of gabapentin drug in the nanohybrid structure.

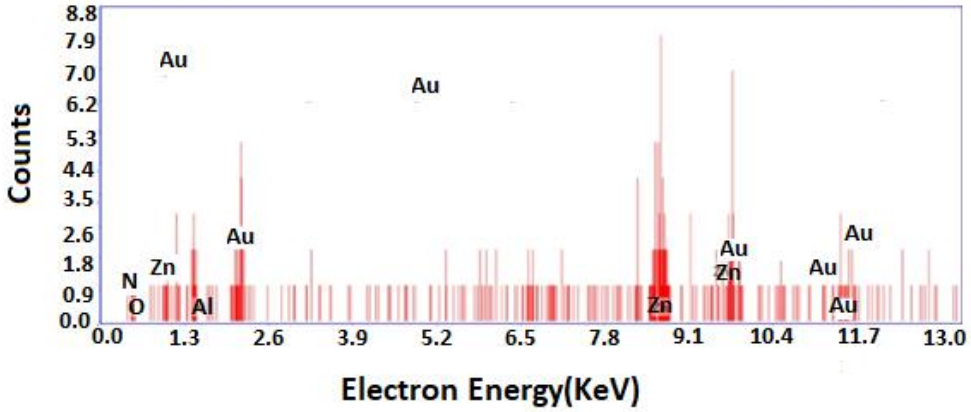

Fig. 5. Energy-dispersive X-ray spectroscopy for Gabapentin-Zn_2Al-LDH

3.5. Gabapentin-Zn_2Al-LDH drug release study

At first, LDH was used as a substrate for the release of gabapentin. Figure 6 shows the release percentage value. As seen in Figure 6, the highest release percentage of gabapentin for the hybrid gabapentin sample is 98%. The release of the drug in the first 50 minutes from between the layers is observed explosively, in the initial moments, this release rate can be attributed to the drugs that were absorbed on the hybrid surface and accumulated in the outer layers. After the intense release of the drug, the release behavior continues in a manner processed by the drug-controlling anion exchange mechanism. In this condition, the anions in the buffer solution are

replaced by interlayer drugs through permeation and anion exchange. The bond these anions create after being placed in the LDH layers is relatively strong. As time goes by and more buffer anions are placed in the hybrid, it becomes more difficult for the drug to leave the hybrid, because the drugs located in the deeper and central parts of the layers have to follow a more complicated path to get out from between the layers. As time passes, the amount of drug released at intervals becomes less and less.

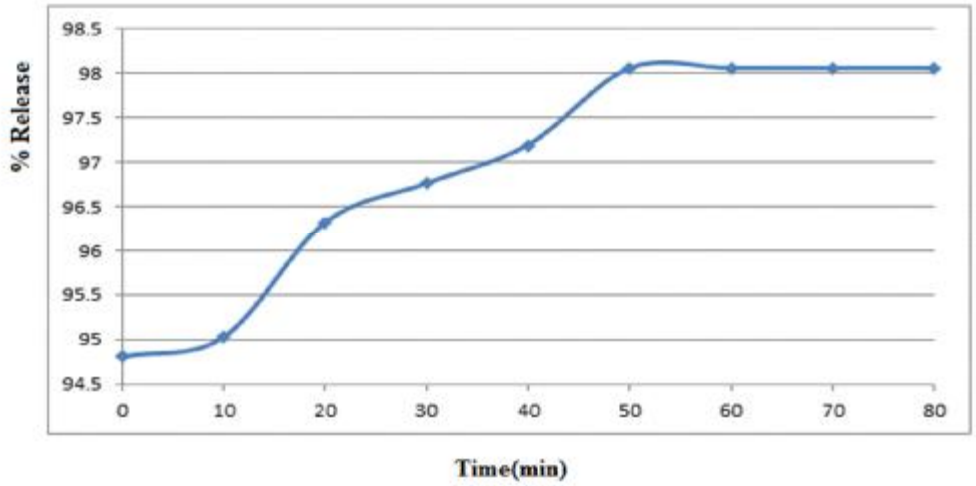

Fig. 6. The percentage of Gabapentin drug release from the Gabapentin-Zn_2Al-LDH nanohybrid

3.5. MTT assay of Gabapentin-Zn_2Al-LDH

According to Figure 7 (a) and (b), 1 M concentrations of Gabapentin-Zn_2Al-LDH in volumes of 125 10^{-3} mL and 500 10^{-3} mL have a high vital efficiency of 68.23 and 58.2 in 48 hours and 70.22 and 49.76 in 24 hours for intestinal cell line cells, respectively. Therefore, Gabapentin-Zn_2Al-LDH has suitable biocompatibility for use in clinical and professional treatment centers.

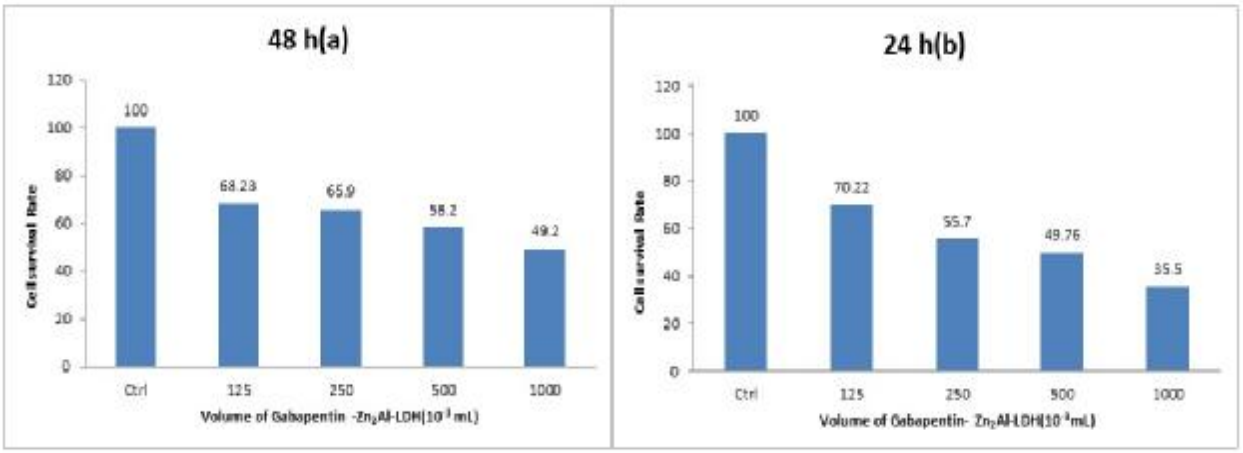

Fig. 7. The Cell Survival Rate of the Gabapentin-Zn_2Al-LDH in 48h(a) and 24h(b)

4. Conclusion

The results show that the synthesis of LDH-gabapentin nanohybrid by co-precipitation method at pH = 8 and temperature of 70 °C gives the best product. The X-ray diffraction pattern shows a high intensity peak of pure LDH with nitrate ions, and the shift of the peak to a smaller angle indicates the intercalation of gabapentin between the LDH layers**.** SEM results show that substituted gabapentin is prepared in the form of nanoscale pebbles between the LDH layers. The drug release results also show that in the simulated environment, 98% of the drug

gabapentin is gradually released from the LDH layer during the first 50 minutes. This nanohybrid can release gabapentin within 1–2 hours in vivo.

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Conflict of interest

There are no conflicts to declare.

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Reference

[1] E.E. Gaskell, T. Ha and A.R. Hamilton, Ibuprofen intercalation and release from different layered double hydroxides. Ther. Deliv., 9(2018) 653-666.

[2] T.A Silva, T.G Nascimento, R.E. Yatsuzuka, L.A.M. Grillo and C.B. Dornelas, Recent advances in layered double hydroxides applied to photoprotection. Einstein (São Paulo), 17(2019).

[3] M. Arif, G. Yasin, L. Luo, W. Ye, M.A. Mushtaq, X. Fang, X. Xiang, S. Ji and D. Yan, Hierarchical hollow nanotubes of NiFeV-layered double hydroxides@ CoVP heterostructures towards efficient, pH-universal electrocatalytical nitrogen reduction reaction to ammonia. Appl. Catal., 265(2020) 118559.

[4] R. Gao, D. Yan, D.G. Evans and X. Duan, Layer-by-layer assembly of long-afterglow self-supporting thin films with dual-stimuli-responsive phosphorescence and antiforgery applications. Nano Res., 10(2017) 3606-3617.

[5] N. Hashim, S.N.M. Sharif, Z. Muda, I. Md. Isa, N.M. Ali, S.A. Bakar, S.M. Sidik and M.Z. Hussein, Preparation of zinc layered hydroxide-ferulate and coated zinc layered hydroxide-ferulate nanocomposites for controlled release of ferulic acid. Mater. Res. Innov., 23(2019) 233-245.

[6] W. Ye, X. Fang, X Chen and D. Yan, A three-dimensional nickel–chromium layered double hydroxide micro/nanosheet array as an efficient and stable bifunctional electrocatalyst for overall water splitting. Nanoscale, 10(2018) 19484-19491.

[7] C.C.F. Meneses, P.R.M. de Sousa, and L.C. Pinto, Layered double hydroxide–indomethacin hybrid: A promising biocompatible compound for the treatment of neuroinflammatory diseases. J. drug del. sci. tech., 61(2021)102190.

[8] Y. Zhao, H. Lin, M. Chen, and D. Yan, Niflumic anion intercalated layered double hydroxides with mechano-induced and solvent-responsive luminescence. Ind. Eng. Chem. Res., 53(2014)3140-3147.

[9] K. Zhang, Z.P. Xu, J. Lu, Z.Y. Tang, H.J. Zhao, D.A. Good and M.Q. Wei, Potential for layered double hydroxides-based, innovative drug delivery systems. Int. J. Mol. Sci., 15(2014) 7409-7428.

[10] V.K.A. Shirin, R. Sankar, A.P. Johnson, H.V. Gangadharappa, and K. Pramod, Advanced drug delivery applications of layered double hydroxide. J. Control. Release., 330(2021) 398-426.

[11] J.H. Choy, S.Y. Kwak, Y.J. Jeong and J.S. Park, Inorganic layered double hydroxides as nonviral vectors. Angew. Chem. Int. Ed., 39(2000) 4041-4045.

[12] V. Ambrogi, G. Fardella, G. Grandolini, M. Nocchetti and L. Perioli, Effect of hydrotalcite-like compounds on the aqueous solubility of some poorly water-soluble drugs. J. Pharm. Sci., 92(2003)1407-1418.

[13] A.L.M.D. de Sousa, W.M. Dos Santos, M.L. de Souza, L.C.P.B.B. Silva, A.E.H.K. Yun and C.S.B.Aguilera, Layered double hydroxides as promising excipients for drug delivery purposes. Eur. J. Pharm. Sci., 165(2021) 105922.

[14] M. Del Arco, A. Fernandez, C. Martin and V. Rives, Solubility and release of fenbufen intercalated in Mg, Al and Mg, Al, Fe layered double hydroxides (LDH): The effect of Eudragit® S 100 covering. J. Solid State Chem., 183(2010)3002-3009.

[15] M.Wei, S. Shi, J. Wang, Y. Li and X. Duan, Studies on the intercalation of naproxen into layered double hydroxide and its thermal decomposition by in situ FT-IR and in situ HT-XRD. J. Solid State Chem., 177(2004)2534-2541.

[16] H. Clarke, R.P. Bonin, B.A. Orser, M. Englesakis, D.N. Wijeysundera and J. Katz, the prevention of chronic postsurgical pain using gabapentin and pregabalin: a combined systematic review and meta-analysis. Anesth Analg, 115(2012)428-442.

[17] E.M. Tiippana, K. Hamunen, V.K. Kontinen and E. Kalso, Do surgical patients benefit from perioperative gabapentin/pregabalin? A systematic review of efficacy and safety. Anesth Analg, 104(2007)1545-1556.

[18] F.A. Rad and Z. Rezvani, Preparation of cubane-1, 4-dicarboxylate–Zn–Al layered double hydroxide nanohybrid: comparison of structural and optical properties between experimental and calculated results. RSC Adv., 5(2015) 67384-67393.

[19] M. Haraketi, K. Hosni and E. Srasra, Intercalation of salicylic acid into Zn-Al and Mg-Al layered double hydroxides for a controlled release formulation. J. Colloid Sci., 78(2016) 533-541.

[20] S. Li, Y. Shen, M. Xiao, D. Liu and L. Fan, Synthesis and controlled release properties of β-naphthoxyacetic acid intercalated Mg–Al layered double hydroxides nanohybrids. Arab. J. Chem, 12(2019) 2563-2571.

[21] A.M.Bashi, S.M. Haddawi and M.A. Mezaal, Layered double hydroxides nanohybrid intercalation with folic acid used as delivery system and their controlled release properties. Arab. J. Sci. Eng., 38(2013)1663-1680.

[22] R. Celis, M.A. Adelino, B. Gamiz, M.C. Hermosin, W.C. Koskinen and J. Cornejo, Nanohybrids of Mg/Al layered double hydroxide and long-chain (C18) unsaturated fatty acid anions: Structure and sorptive properties. Appl. Clay Sci., 96(2014) 81-90.

[23] T.G. Mayerhöfer, S. Pahlow and J. Popp, The Bouguer-Beer-Lambert law: Shining light on the obscure. ChemPhysChem., 21(2020) 2029-2046.

[24] L. Tolosa, M.T. Donato and M.J. Gomez-Lechon, General cytotoxicity assessment by means of the MTT assay. Protocols in in vitro hepatocyte research, (2015) 333-348.

[25] S.J. Santosa and E.S. Kunarti, Synthesis and utilization of Mg/Al hydrotalcite for removing dissolved humic acid. Appl. Surf. Sci., 254(2008)7612-7617.

[26] I. Fleming and D. Williams, Infrared and raman spectra. Spectroscopic methods in organic chemistry, (2019) 85-121.
[27] F.A. Rad, Z. Rezvani and F. Khodam, Molecular design confirmation for proposition of improved photophysical properties in a dye-intercalated layered double hydroxides. RSC Adv., 6(2016) 11193-11203.