Biological activitity of nano hybrid from Rifampicin intercalated with Ni/Al and Mg/Al Layered double hydroxides

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Abstract:

This work included synthesis of Rafampicin intercalated with both Mg/Al and Ni/Al Layered double hydroxide to give a nano hybrid materials, these materials were characterized by X-Ray diffractions (XRD) and Fourier transformation Infra red(FTIR), controlled release and Biological activity tests of the resulted nano materials was evaluated against both gram positive and gram negative bacteria.

النشاط البيولوجي لمركب نانوي من الرافمبسين المجسر لمركبات ثنائية الهيدروجين من نيكل\المنيوم و مغنسيوم\المنيوم

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المفتاح:الرافمبسين, طبقات ثنائية الهيدروكسيل مركبات نانوية

الخلاصة:

هذا العمل يتضمن تخليق الرافبمسين المجسر لطبقات ثنائية الهيدروكسيل متكونة من مغنسيوم\المنيوم و نيكل\ المنيوم ليكون مواد ذات تهجين نانوي تم تشخيصها بواسطة حيود الاشعة السينية وطيف الاشعة تحت الحمراء, درست السيطرة على تحرر الدواء من كلا الطبقتين وحدد الموديل الرياضي الذي ينطبق على سير التحرر كذلك اجريت الفحوص البيولوجية التي تخص النشاط البيولوجي على نوعين من البكتريا الموجبة والسالبة وعينت نسبة النشاط البايولوجي

Introduction

Nanoparticles have been widely used in optical, resonant, electrical and magnetic fields, etc. Various chemical methods have been used for the production of nano particles with narrow size distribution such as micro emulsion method, electro spray pyrolysis and hydrothermal methods [1–5]. Layered double hydroxide (LDH) commonly known as hydrotalcite-like materials or anionic clays are a family of natural and synthetic materials with general formula $[M2+ x M3+ y (OH)2]x+An− x/n \cdot yH2O$, where M2+ are divalent cations, M3+ are trivalent cations, An− is the interlayer anion and x is the mole fraction of M3+/(M2++ M3+).The positive charge of the layer is compensated by anions, which occupy the interlayer space along with water molecules[6] A wide range of anions could be intercalated for the formation of LDH-intercalated or the so-called the host guest type materials. The LDH can be regarded as the layered host which hosted the guest anions make up part of the negatively charged sheets of the LDH. If the anion is of beneficial agents such as drugs or pharmaceutical compounds are of the ion-exchange properties of LDH, then this type of materials can be exploited as a controlled release formulation (CRF).Such works had been carried out for plant growth regulator,[7] herbicide[8] biomolecules,[9] therapeutics such as ibuprofen,[10]

camptothecin,[11]-fluorouracil,[12] and others. It may also be particularly useful for DNA and ATP storage due to higher stability of the biological materials in the LDH interlamellae compared to their counterparts.
Rifampicin is one of the most potent and broad spectrum antibiotics against bacterial pathogens and is a key component of anti-tuberculosis therapy, stemming from its inhibition of the bacterial RNA polymerase(RNAP)[13].
The aim of this study was to prepare layered double hydroxide using Ni/Al and Mg/Al intercalated with rifampicin because this type of nano hybrid compounds have attracted great attention in many application fields.

2.0 Materials and Methods

2,1- Method:

Capsules Bp 300 mg of rifampicin ($C4_3H_{58}N4O_{12}$, molecular weight 822.95 gm/mol) was purchased from pharmaceutical co. ltd , (S.D.I – IRAQ) . Al(NO3)3.9H2O (98%) was purchased from xinbao nice chemical .,NaOH and Mg(NO3)2.6H2O (98%) were purchased from xilong chemical , Ni(NO3)2 were purchased from Merk. All chemicals were of analytical grade and used without further purification. Layered double Hydroxides(LDH) were prepared as follow:- molar ratio of Ni/Al and Mg/Al= 4 in 100ml deionized water with total concentration of 0.1M for each type LDH.

NaOH of 2M was dropped wise to above solution until the pH adjusted at 10.5 with continuous stirring for two hours, this mixture was left for 18hrs at 30 oC for aging then centrifuged at 3500 rpm and washed four times with deionized water.

Precipitations (ppt) was collected and dried, 0.5gm of the ppt was putted in 50ml de ionized water and subjected to stirring with magnetic stirrer. 300ppm of rifampicin was prepared in 50ml de ionized water, dropped during one hour on the LDH suspension, left to aging for 18hrs at room temperature then centrifuged washed four times by de ionized water, dried at 30 oC and stored for further uses. controlled release was done in solution of 0.5M $CaCO_3$ adjusting the rifampicine concentration by Uv-Vis Spectrophotometer 7200 Cecil using the lambda max.

Nutrient agar, Muller Hinton broth and Muller Hinton agar were purchased from Himedia. Antibacterial activity of rifampicin nano particle compared with the free one was studied by agar well Diffusion method (14). Four bacterial isolates were obtained from biology department, college of science- university of Kerbala and used in this study. The isolates included two gram positive(Staphyllococcus aureus and Streptococcus fecalies) and two gram negative (Escherichia coli and Pseudomonas aeruginosa). The cultures of the isolates were prepared by transferring a loop full

of bacteria from nutrient agar slants into Muller Hinton broth and incubated at 37 ^{o}C for 18 hrs. 100µl of each grown isolate was spread on sterile Muller Hinton agar plates. Wells(5mm) were cut from the agar with sterile borer and 40µl of the rifampicin nano particle(dissolved in DMSO) was added to each well. Following incubation at 37 ^{o}C for 24hrs, diameters of the inhibitory zones were measured.

3. 2 Characterizations

Powder X-Ray Diffraction patterns (PXRD) were obtained with a Shimadzu XRD-6000 powder diffractometer using (λ=1.540562 Å) at 40 kV and 30 mA a scan rate of 2 min./degrees

Fourier Transform Infrared (FTIR) spectra were recorded by using a spectrophotometer thermo Nicolet Ft-IR Nexus self supporting sample in the range of 4000-400 cm-1.

3.3: Results and discussion

Fourior Transformation Infra Red(FTIR) spectra of the drug–LDH hybrid, compared with that of the pristine LDH, are in some way different as shown in Fig.[1] The broad band at 3387 cm-1 can be assigned to stretching vibration of the N-H (15) , . the bands in the range of 2980- 2880 cm-1 are attributed to C-H stretching vibration of rifampicin anion [16] . The stretching vibration peak at 1354 cm-1 (due to $CO3^{2-}$ converted from CO_2 captured from air during washing) [17] . The intercalation of the counter anion (NO3-) can be ruled out based on the absence of the absorption peak ant 1384 cm-1 [18] . The band at 1712 cm^{-1} attributed to the C=O group of acetyl stretching at the same time , and it has a higher wave number than those of due to the strong intercalation between surface layers and carboxyl group of rifampicin , while bands at 1620 cm^{-1} which is attributed to the C=O of amid group , and finally, the bands at 1567 cm-1 and 1485 cm^{-1} are assigned to the stretching vibrations of C=C in the back bone of aromatic ring . The band 1461 cm^{-1} which is attributed to the –C-N stretching , C-C the band of (1290-1021) cm^{-1} , which is attributed to the C-O-C acetyl group , the band 2781-2682 cm^{-1} which is attributed to the CH3-N stretching , the band 2894 cm-1 which is attributed to the CH_3-O asymmetric stretching , the band 2926 cm^{-1} which is attributed to the group - CH_3 stretching [19] . Moreover , in the low – frequency region , the band from M-O and O-M-O (M=Mg ,Ni, Al) groups appear in the 400 – 800 cm^{-1}

Fig.2 FTIR spectrum of LDH and RiF- LDH, nanocomposite

3,3.1: X-Ray Diffractions :

the solids obtained that indicate after hydrothermal treatment at 120 a Ni-Al-NO3-LDHs nanohybrids have been formed "Figure[4] respectively, (009), respectively. For the hydrothermally treated sample Ni-Al-$NO3^{-}$

Fig.4. XRD of Ni/Al LDH(black) and Ni/Al-Rifampicine shows the different diffractions.

LDHs at 120˚C the basal reflections are recorded at 1.6nm(003), 1.2nm(006), and 0.7 nm(009) respectively.. Powder XRD patterns of the Ni-Al-NO3-LDHs samples showed that the full width

of half maximum (FWHM) value of (003) diffraction line decreased with increasing temperature of hydrothermal treatment, which indicated an increase of the LDH crystallite size. In addition, the intensity of (003) plane was increased with increased the temperature as can be seen in "Fig[4]. For both samples Ni-Al-rifampicin-LDH.

Fig 3 shows the xrd of Mg/Al-LDH for d(003), d(006) of 0.8 and 1.4nm respectively in the same time the Mg/Al- rifampicine nano hybrids basal reflections are recorded at 1.4 (003), 0.7 (006), and 0.35 nm (009) respectively. That give an indication of crystal homogeneity in the case of Ni/Al LDH is better than in the case of Mg/Al LDH.

Fig.3. XRD of Mg/Al -LDH and Mg/Al-Rifampicin shows the different diffractions.

Controlled released:

It was observed that carbonate dominated the accumulated release percentage as shown in (Table 1, 2) due to, carbonate was known to have the strongest affinity toward the interlayer of layered double hydroxides, also the release rate of Rifampicine(Rif.) in the carbonate solution was found to be rapid.

A rapid release of Rifampicine occurs at the initial stage, which is followed by a slower release of (Rif.). As shown in, (Rif.) is almost 88% replaced by $CO3^{2-}$, resulting in the highest accumulated release among the media studied. The maximum release time shows that (Rif.) in $CO3^{2-}$ media was achieved with 435 min.:

$C_t/C_o=Kt$ ------zero order module

$-\log(1-C_t/C_o)= \log /kC_e - kt$ -------1st order module

$t/C_t=1/kC_e^2+1/C_e\, t$ --------2nd order module

$-\log(1-C_t/C_o)= t^{0.5}$ bashker equation

Aqueous solution	Concentration (Mol.L-1)	Maximum Release %	Maximum Time (min)	Zeroth order	First order	Bhaskar equation	Pseudo -second order	Other parameters for pseudo-second order	
				r2				K×10-4 (L.mg-1 min-1)	t1/2 (min)
CaCO3	0.500	88	435	0.696	0.818	0.914	0.997	370.000	10.366

Table 1. Percentage Release , Rate constant (K), Half life (t1/2) and correlation coefficients (r2) obtained from fitting of the release Data of (Rif) from Mg-Al-Rif-LDH nano hybrids in to various aqueous solution (Mg/Al=4- ion exchange).

Aqueous solution	Concentration (mol.L-1)	Maximum Release %	Maximum Time (min)	Zeroth order	First order	Bhaskar equation	Pseudo second order	Other parameters for pseudo second order	
				r2				K×10-4 L.mg-1 . min	t0.5 (min)
CaCO3	0.5	90	2780	0.793	0.895	0.842	0.995	1.9	36.46
	0.005	95.5	2780	0.816	0.968	0.972	0.995	17	7.225

Table2. Percentage Release , Rate constant (K), Half life (t1/2) and correlation coefficients (r2) obtained from fitting of the release Data of (Rif) from Ni-Al-Rif-LDH nano hybrids in to various aqueous solution (Ni/Al=4- ion exchange).

We can say that the release to the media of carbonate from Ni/Al-Rif- nanohybrids is more easy than the release from Mg/Al-Rif- nanohybrids as table 1 and 2 shows this due to the different interactions between rifampicine and the two different LDH.

Biological activity of Rifampicin-LDH:

The results of the antibacterial activity of rifampicine nano particle are presented in table 3.

The results have shown that both gram positive and gram negative bacteria were sensitive to the rifampicin as free molecule whereas only. S. aureus was sensitive to the rifampicine nano particle.

The rifampicin free exhibited highest anti S. aureus activity with an inhibition zone of 36mm at 500µg/ml antibiotic concentration. The results have also shown that rifampicine nano particle exhibited less anti S. aureus activity in comparing with inhibition zone against the free one, and the Ni/Al-Rif. gave the lowest inhibition zone against the above bacteria.

The minimum inhibitory concentrations of rifampicin were (1,50,50 and 10) µg/ml against the bacterial isolates (S. aureus, Streptococcus fecalis, E.coli and P.aeruginosa, respectively while the Mics of Mg/Al – and Ni/Al- rifampicin

were(10and 50) µg/ml against S.aureus, respectively.

Rifampicine contain an aromatic nucleus linked on both sides by an aliphatic bridge . Rifampicine diffuses freely into tissues, living cells, and bacteria making it extremely effective against intracellular pathogens. Their bactericidal activity has been attributed to their ability to inhibit transcription by binding with high affinity to bacterial DNA-dependent RNA polymerase(20,21).

Type of antibacterial	antiantibiotic Concentration (µg/ml)	Inhibition zone (mm)			
		Staph. aureus	Strep.fecalis	E.coli	P.aeruginosa
Rif. free	500	36	20.7	15.5	22.5
	100	30.5	13.5	12.5	20.5
	50	28.5	7	10	18.5
	10	27.5	0	0	11.5
	1	23.5	0	0	0
Mg/Al-Rif.	500	22.5	0	0	0
	100	16.0	0	0	0
	50	14.5	0	0	0
	10	10.5	0	0	0
	1	0	0	0	0
Ni/Al-Rif.	500	18	0	0	0
	100	7	0	0	0
	50	5.5	0	0	0
	10	0	0	0	0
	1	0	0	0	0

Fig. 3. Represent the results of the antibacterial activity of rifampicine nano particle.

4.0:References:

[1] Y.H. Zheng, Y. Cheng, F. Bao, Y.S. Wang, Mater. Res. Bull. (41) 525–529 2006.
[2] M. Takagi, T. Maki, M. Miyahara, K. Mae, Chem. Eng. J. (101)269–276, 2004.
[3] J. Wagner, T. Kirner, G. Mayer, J. Albert, J.M. Ko¨hler, Chem. Eng. J. (101)251–260, 2004.
[4] Y.J. Suh, H.D. Jang, H.K. Chang, D.W. Hwang, H.C. Kim, Mater. Res. Bull. (40), 2100–2109, 2005
[5] F. Sayılkan, S. Erdemoglu, M. Asilturk, M. Akarsu, S. Sener, H. Sayılkan, M. Erdemoglu, E. Arpac, Mater. Res. Bull. (41),2276–2285,(2006).
[6]. M.Z.Hussein, Z.Zainal, and A.Y ahaya, J. Control Rel. (82), 417, 2002.
[7]. M.Z.Hussein, Z.Zainal, A.Y ahaya, and H.K.Loo, Sci. Technol. Adv. Mater. (6), 956, 2005.
[8]. J.H.Cho y, S.Y .Kw ak, Y.J.Jeong, and J.S.P ark, Angew. Chem. (39), 4041,2000.
[9] V.Ambrogi, G.F ardella, G.Grandolini, and L.Perioli, Int. J. Pharma. (220), 23, 2001.
[10] K.M.T yner, S.R.Schiffman, and E.P .Giannelis, J. Control Rel. (95), 501, 2004.
[11] Z.W ang, E.W ang, L.Gao, and L.Xu, J. Solid State Chem. (178), 736, 2005.
[12] D. Pan , H. Zhang , T. Zhang , X. Duan , ," Chem. Engineering Sci. (65) 3762-3771,2010, .

[13] Yu-Jen Chen , Materials Research Bulletin (46)1922-1927,2011, .
[14] NCCLS, (National Commite for Clinical Laboratory Standard). Performance Standards for Antimicrobial Susceptibility Testing. 9 th. International Supplement. M100-S9, Wayne Pa, 1999.
[15] P. Gunawan ,RONG XU , Received 4 september 2007 , accepted 17 December 2007 published online in wiley inter science
[16] Longchao Du , Baojum Qu , Yuezhong M , Quan Zhu, Composites science and Technology (6913-9186), 2006.
[17] Mohammed Adnan M, M.Sc thesis University of Kerbala / college of science 2012.
[18]- Yu-Jen Chen Athesis submitted in fulfillment of the requirements for the degree of master of science of Rhodes university (2000).
[19] Eliz. A. Campbell, N. Korzhera, A. Mustaev, K. murakami, S. nair, A. Goldfarb and seth A. Darst" Cell.(104),901-912,March 23,2001, copyright ©2001 by cell press.
[20] Kolyva,A.S. and Karakousis, P.C.. Johns Hopkins university Center for Tuberculosis Research. Baltimore, M.D., USA.www.interchopen.com , 2013.
[21] Shinnick, T., 1996. Current Topics in Microbiology and Immunology(New York: Springer_V

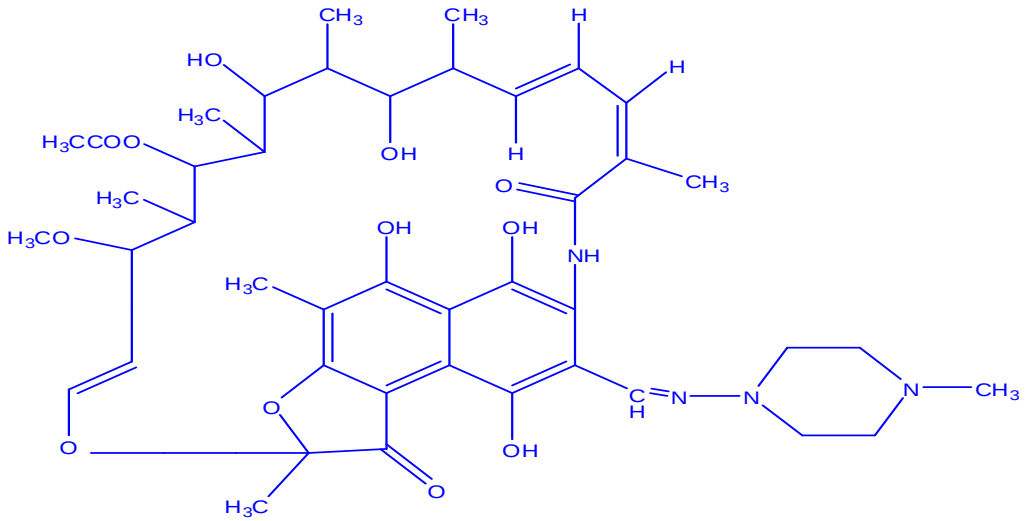

Fig. 1. Molecular structure of the drug rifampicin

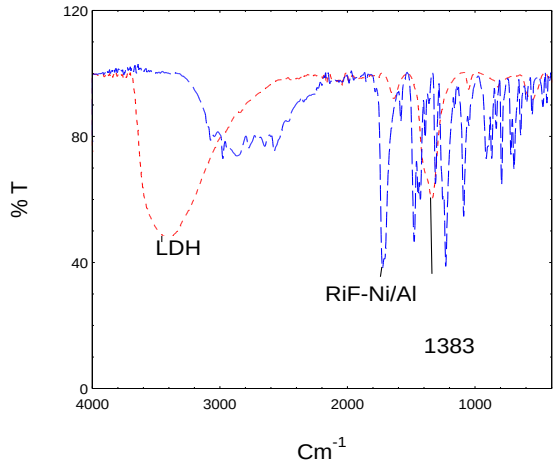

Fig 2: Ftir spectrum of LDH and LDH-Rimpicine nano composite

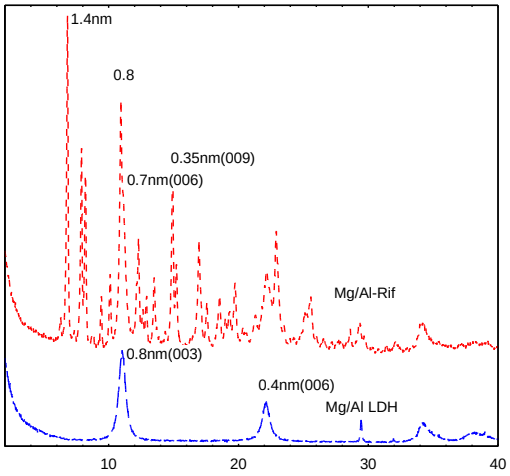

Fig.3. XRD of Ni/Al LDH(black) and Ni/Al-Rifampicine shows the different diffractions.

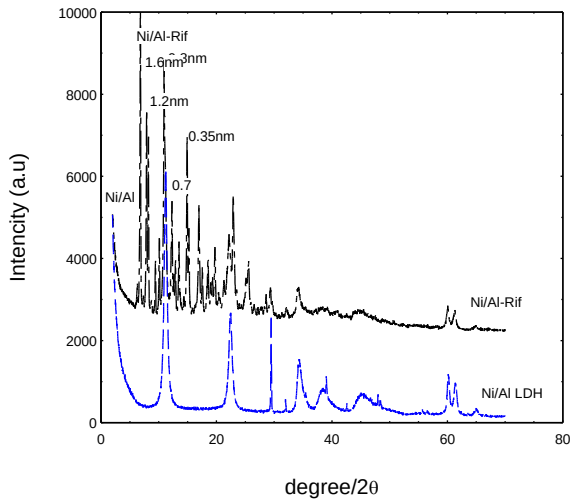

Fig.4. XRD of Mg/Al -LDH and Mg/Al-Rifampicin shows the different diffractions.