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Characterization of silver nanohybrid with layers double hydroxide and demonstration inhibition of antibiotic-resistance *Staphylococcus aureus*

Eussur N. Alkhafaji[a], Noor A. Oda[a] and Luma M. Ahmad*[b]

[a,]College of Pharmacy,University ofAhl AlBayt, Karbala, Iraq
[b] Department of Chemistry, College of Science, University of Kerbala, Karbala, Iraq
Yosornoory@gmail.com
abdalamernoor@gmail.com
*luma.ahmed@uokerbala.edu.iq

Abstract

Work aimed to focus on the synthesis of the nanohybrid compounds from silver nanoparticles (AgNPs) with Nickel/Aluminum layers of double hydroxide (LDHs) by the direct and indirect ion-exchange method at pH 6.5 as a light green-yellow colored nanostructure powder. The silver nitrate precursor was reduced to Ag nanoparticles by starch as reducing and coveringagents solutions at pH 8. The resultant silver nanoparticles are integrated on LDHs surface which is loaded on its surface for 12h. These nano compounds were characterizeby X-Ray Diffraction, Atomic Force Microscopy, Fourier Transform Infrared spectra, and UV-Visible spectrophotometer. The XRD data and AFM images demonstrated that the prepared AgNPs, LDHs, and AgNPs with LDHs are nanomaterials with nano-spherical, and nano- rod and nanorod too, respectively. The antibacterial Performances of Ag-NPs-Ni/Al–LDH manufactured by direct and in direct Ionic Exchange method , Ag NPs were determined against *Staphylococcus aureus* bacteria. So at the 4th recycled used, the kill percentages of Ag NPsNi/Al–LDH – direct was still as higher as 100%. The growth behaviors of *S. aureus* were checked by the visual density at 600nm (OD600). These anew designed of (Ag-NPsNi/Al–LDH) layers might a promising antibacterial solution as alternative drugs to antibiotics for clinical and environmental applications.A qualitative assessment of the anti-biofilm formation effect was performed using a modified test tube method by measuring the optical density. The results demonstrated the efficient antibiofilm activity of Ag NPsNi/Al–LDH – with the direct method, its highest compared with other studied molecules and the antibiotics itself. The current data highlights, biogenic NPsNi/Al–LDH – with direct method could be used as an adjuvant for antibiotics and anti-biofilm formation in the treatment of bacterial infections.

Keywords:Nickel/Aluminum layers of double hydroxide (Ni/Al-LDHs);AgNPs; Ag NPsNi/Al–LDH; Nano-rod; Nano-spherical;antibiofilm ;*Staphylococcus aureus***.**

1. **Introduction**Nanoscale materials show unique properties that cannot be practical in other compounds that are due to them. The reason for this is that the size and form of nanoparticles when they are on the nanoscale scale alter the chemical and physical properties of the compound itself. Also, silver nanoparticles (Ag NPs) provide a lot of attention that values their ice salt potential. Because of its diversity in its applications in many fields [1]. Including antibacterial agents due to the severity of toxicity towards the microbes while it decreases its toxicity towards the animal cells, and the high ability to deliver drugs, as used as chemical stimuli and photoelectrons, and also used in optical sensors [2]. Also, the silver nanoparticles have a large specific area, which makes them suitable as catalysts[3]. More recently, it has found its ability to kill cancerous tumors, meaning that it works as anti-cancer agent[4]. The collection of silver nanoparticles is one of the main reasons that reduce their activity, so their surface can be modified by inserting them between the two layers of hydroxide to increase their efficiency in many previous applications[5]. Due to the progress made by LDH nanostructures in many fields because they possess great possessions for example easiness of marked, and the capability insert unalike types ions (inorganic, organic, biomolecules, genetic). LDHs

*Corresponding author e-mail: luma.ahmed@uokerbala.edu.iq; (Luma M. ahmed).

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than like -hydrotalcite or clays anion of loads (^{+}OH) sheets and interlayer charge matching anions. Construction LDHs container describes seeing the construction of Magnesium hydroxide in which the Magnesium ions are octahedral coordinated to (^{-}OH) groups[6].The interplanetary between the octahedral(e.g., M^{2+}: Mg^{2+}, Co^{2+}, $Zn^{2+,}$ and M^{3+}: Al^{3+}, Ga^{3+}, Fe^{3+}, Mn^{3+}, Gd^{3+} etc.) layers might be full via intercalated anions vitamins[7]. DNA, insecticide [8], and drugs, with water in residence by hydrogen bonding to the hydroxyls, via the pharmacokinetics, toxicity, and therapeutic effectiveness LDHs take described[9]. In position the Nickel/Aluminum-LDH phase contest with polymeric and inorganic, it is significant to express the potentia for added modalities with approval to therapy and imaging[10]. Nickel/Aluminum-LDH intricate big attention owing tothe possible requests in controlled sluggish delivery and release requests [11-12].Also combination of nanocomposite inorganic-organic compounds [13-14]. At this time, information on the formation and explanation of a composite AgNPs-Nickel/Aluminum-LDH nano compound hybrid prepared via ion exchange method and the determination for education increase solidity of the Ag nanoparticle per Nickel/Aluminum-LDH matrix. The study of the anti-bacterial Nano Particles is very significant for the growth of more actual anti microbial materials.
This study aims to prepare hybrid nanocomposites by combining silver nanoparticles with Nickel/Aluminumlayers double hydroxide through direct and in direct Ion-Exchange methods to increase the efficiency of bacterial growth inhibition and anti-biofilm.The results showed that the direct method of preparing nanocomposites is the most efficient in inhibiting bacterial growth

2. Experimental

A. Preparation of Nickel/Aluminum-LDHs

The ordinary layer double hydroxyl was prepared through the Co-precipitation method. The exact molar ratio 2:1 was prepared using 0.1M of Nickel nitrate and 0.05M of Aluminum nitrate. At temperature 298K and pH (6.5), 2M sodium hydroxide solution was slowly added to theformed Nickel/Aluminum-LDHs solution with moving for 12 hours. The dispersed precipitate was weekly washedwith distilled water(D.W) and airing at room fever[8-15].

B. Preparation Ag PNs

3mM of AgNPs was prepared via dissolved 0.015g of silver nitrate in mixing from 20 mLD.Wwith 10 mLethanol. The reduction reagent (2% starch solution) was dissolved in 20 mL D.W. The starch solution was added to Ag^{+} solution with moved usinga magnetic barfor 1 hourat 60-80°C at pH=8. The change in color from colorless to greenish-yellow has happened that indicated toreduce the silver ion (Ag^{+}) to the silver atom (Ag^{0}) using a starch solution.

C. The nanohybrid composite Ag NPS Nickel/Aluminum-LDHs prepare via two methods

C.1. Ion Exchange Indirect

A molar ratio 2:1 was produced when 2.9g of Nickel nitrate with 1.8g Aluminum nitrate were dissolved in 100 mLof ethanol, respectively.ThepH ofthe mixture was fixed at pH 6.5added 2MNaOH drop by drop to Ag^{+} solution. ThepreparedAgNPs solutewas transferred in a water bath with shakingon100ºC 12 hours.

C.2. Ion Exchange Direct

A 0.5g of prepared Nickel/Aluminum-LDHs was dissolved in 50 mLethanol.The prepared AgNPs solution was droppedby drop added and then transferred in water bath with shaking at 100 ºC for 12 hours.

Not: Nanocomposite was prepared using the direction exchange at different pH= 12,10 and 6.5.The method that succeeded in combating bacteria was with pH=6.5

3. Characterization techniques

The physic-chemical possessions of nanocomposite hybrid AgPNs now Nickel/Aluminum-LDHs are imperative used for actions, care, biodistribution, effectiveness, and assess the useful features. The description ofthe Ag NPs- Nickel/Aluminum-LDHs nanohybrid materials via several analytic techniques, counting, Fourier transforms infrared spectroscopy (FTIR), X-ray diffractometry (XRD), and atomic force microscopy (AFM).

A. UV-Visible spectrophotometric

Visual possessions for found yellow color Ag nano particle solution were checked on a UV-Vis spectrophotometric.

B. Fourier Transform Infrared Spectroscopy (FTIR)

The chemical construction and practical group accountable Nickel/Aluminum-LDHs and Ag NPs-Nickel/Aluminum-LDHs nanohybrid was also examined FT-IR spectroscopy in wavelength 4000–400 cm^{-1} at room fever.

C. X-ray diffraction (XRD)

XRDanalysis was applied in ranges10º–80º. It offers material on atom phases,crystal structures, favored feel, and other structural parameters, such as grade of crystallinity, regular modicum size, strain, and crystal flaws.

D. Atomic Force Microscopy (AFM)

Usage the AFM study Nickel/Aluminum-LDHs and Ag NPs- Nickel/Aluminum-LDHsnanohybrid composite dimension of diameter, and gatherings nanomaterials. the replicas were directed to the College of the Science University of Baghdad for the determination of inspection.

E. Antibacterial Performance Test:

This study was conducted to determine the inhibitory efficacy of hybrid nanoparticle silver particles manufactured by direct and in direct Ionic Exchange method against *Staphylococcus aureus* bacteria obtained from the specialized messenger laboratory approved by the Ministry of Health, holy Karbala, and diagnosed by VITEK system.Antibacterial test was conducted according to the procedure described in reports [16-17].

S. aureus wascultivated to aexponential phase in Luria –Bertania broth (L.B. broth) at 37 °C. The cells were collected by sedimentation in centrifugation, washed with phosphate buffer saline (PBS) at pH = 7.2 and re-suspended in PBS with the cell concentration approximately at 10^5 colony forming units (CFUS) per mL.In different tubes were immersed in the cell suspensions (10 mL) 50 µL of the hybrid nanoparticle (Ag NPs-Nickel/Aluminum-LDHs) marked via the direct and indirect method of concentration 0.5 mg/mL.For control, Nickel / Aluminum layer double hydroxide and silver nanoparticles were used.

The mixture was incubated at 37 °C with a gentle shaking at 210 rpm. After 3 h of incubation, using the colony counting method, the number of viable cells were detected. The murder percentage of bacteria was calculated by ($N_{control}$) –(N_{sample}) / ($N_{control}$) X 100% , where $N_{control}$ and N_{sample} represent the number of viable cells in the cell suspensions with control and the sample, respectively .

To evaluate the durability of (Ag NPsNi/Al–LDH) assembled by direct and indirect Ionic Exchange method , the sample was reused for the antibacterial efficacy test under the same conditions for three times. The sample after each test was washed with both PBS twice and sterilized distilled water .

F.Growth Inhibition Study of Ag NPs-Nickel/Aluminum-LDHs **:**The *Staphylococcus aureus* bacteria was cultured to a mid- exponential phase in LB broth and re-dispersed in LB broth (10 mL) after washing by sterilized distilled water to obtain a concentration of 10^5 CFUs mL-¹.

The samples were then immersed in the suspension of bacteria and incubated at 37 °C with concussion of tubes at 200 rpm.The growth behavior of bacteria was monitored by measuring the optical density at 600 nm (O.D.600) based on the cloudiness of thesuspension using Ultraviolet -vis spectroscopy (Shimadzu -Ultraviolet 2450)[17-18].

G. Anti-Biofilmformation Test of Ag NPs-Nickel/Aluminum-LDHs **by Tube method (TM):***Staphylococcus aureus* in the mid-exponential phase was mixed with 4 ml of tryptic soy broth (TSB) at 10^5 CFUs per mL and 100µl from 50 mg/ml stock of the hybrid nanoparticle Ag NPs- Nickel/Aluminum-LDHs marked via the direct and indirect methods , Nickel / Aluminum layer double hydroxide and silver nanoparticles .

The suspension in the tube was cultured at 37 °C with concussion and shaking at 100 rpm. After 48 h culture, the samples were washed gently with al PBS and stained with crystal violet dye for a 1 minute. And then, the unbounded dye was removed with PBS and the dye absorbed by the biofilm was retrieved with acetic acid and its density was evaluated using a spectrophotometer at 595 nm[19-20].

4. Results and Discussion

A. UV-Vis- spectrophotometric

Figure1 estimates the visible effects of silver nanoparticles occurs at the highest absorption of the particles at the wavelength equal to 450 nm and that the high absorption value indicates to produce the large size of the silver nanoparticles, so, the color change from colorless to yellow and then to green-yellow that due to formation of silver nanoparticles [21-22].

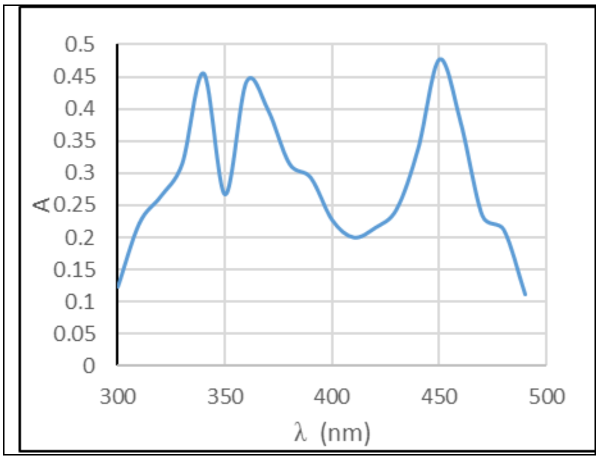

Fig. 1. TheVisible spectrum AgNPs solution.

B. FTIR- spectrum

Figures (2-5) carry out the FTIR spectrum of Ag nanoparticles, Nickel/Aluminum– NO_3-LDH, and Ag NPs-Nickel/Aluminum-LDHs, respectively.

FT-IR spectroscopy was employed to estimate the interactions between the functional groups of starch and AgNPs. Figure 2 shows the FT-IR spectra of starch(a)and FT-IR of AgNPs that prepared in present starch that acts as capped agent in 2 % (w/v). At 3305 cm^{-1} , a strong band was observed that due to the $\upsilon_{O–H}$ stretching of starch. An asymmetric stretching of $\upsilon_{C–H}$ band was demonstrated at 2930 cm^{-1}, and the band at 1643 cm^{-1} was attributed to water adsorbed in the amorphous region of starch [23]. The observed characteristic band at 1085 cm^{-1} was due to C–O–H bending of starch. The shift at 3305 and 1085 cm^{-1}band to 3295 and 1076 cm^{-1}, respectively, it was observed in the presence of nanoparticles[24]. The peaks were also broader and longer in the starch capped nanoparticles as compared to starch alone, which might be due to inter and intra-molecular hydrogen bonding. These starch characteristic bands included in the synthesized nanoparticles that confirmed the successful binding of the OH group of starch with AgNPs, absorption groups at 1335 cm^{-1} returning to the (NO_3) group in silver nitrate. Asymmetric ethereal (C-O-C) oscillations are obtained at 1149 and 996cm^{-1}, respectively.Whereas feeble absorption top of δ_{C-H} aliphatic at 726 $cm^{-1}$and that make in an indirect and direct correspondingly and absorbent top cyclδ_{C-H} at 861 cm^{-1} [25].

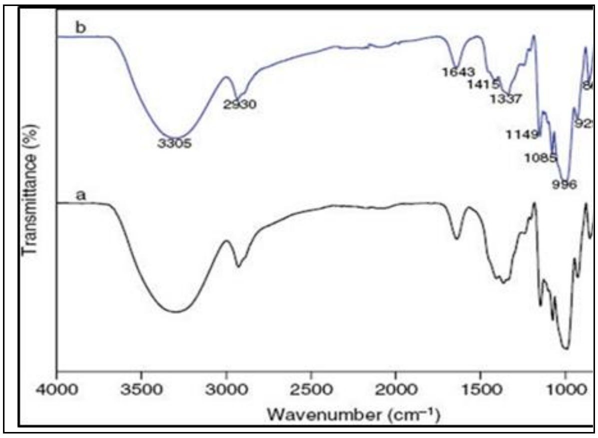

Fig. 2. FTIR spectra of a) starch and b) AgNPs with starch .

Thefig. 3 andTable 1 demonstrate theNickel/Aluminum-LDHs appear the assimilation top $\upsilon_{O–H}$ collection stretches at 3444 cm^{-1} and feeble assimilation top δ_{-OH} at 1639.5cm^{-1} [26]. The NO_3 assimilation top finds at 1386.6cm^{-1} in the layers [27].Assimilation top$\upsilon_{Ni–O}$ at 408.1cm^{-1} in layers and assimilation top$\upsilon_{Al–O}$ at 621.1cm^{-1}in layers [28]wereshown.

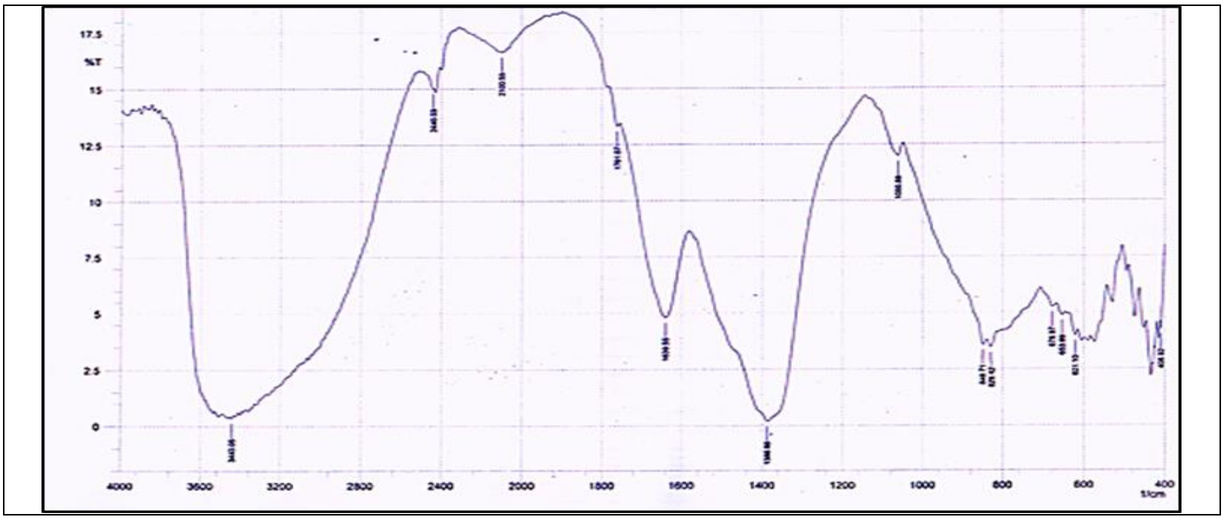

Fig. 3. FTIR for Nickel/Aluminum-NO_3-LDH.

Fig. 4 obtains the nanohybrid composite AgNPs Nickel/Aluminum-LDH looks several a new top this positive aimed at intercalation of Ag Nanoparticles between the layers, top of alkyl $\upsilon_{C–H}$ 2942 cm^{-1} and 2932cm^{-1}in prepared direct and indirect correspondingly. The top cycle $\upsilon_{C–H}$ 1154cm^{-1} and 1150cm^{-1} atprepared direct and indirect correspondingly. Whereas feeble absorption top of δ_{C-H} aliphatic at 706 cm-1 and 703 cm-1 the make in an indirect and direct correspondingly and absorption top cycleδ_{C-H} at 828 and 835 cm-1that make direct and indirect correspondingly, extensive absorption top (3496 $cm^{-1}$and 3427cm^{-1}) is $\upsilon_{O–H}$ and feeble absorption top of δO-H at 1638cm^{-1} and 1641cm^{-1} [29] for prepared direct and indirect correspondingly. The NO_3 stretches a top at 1380 cm-1 in the layers, two top of υC-O-C at 1084 cm^{-1} and 1049 cm^{-1} in making indirect but the two peaks of υ_{C-O-C} at 1083 cm^{-1} and 1017 cm^{-1} [30] in prepared direct. Top to $\upsilon_{Ni–O}$ at 433 $cm^{-1}$and 439cm^{-1} in layers and top $\upsilon_{Al–O}$ at 564$cm^{-1}$and 607cm^{-1}in layers[28], asshown in table 1.

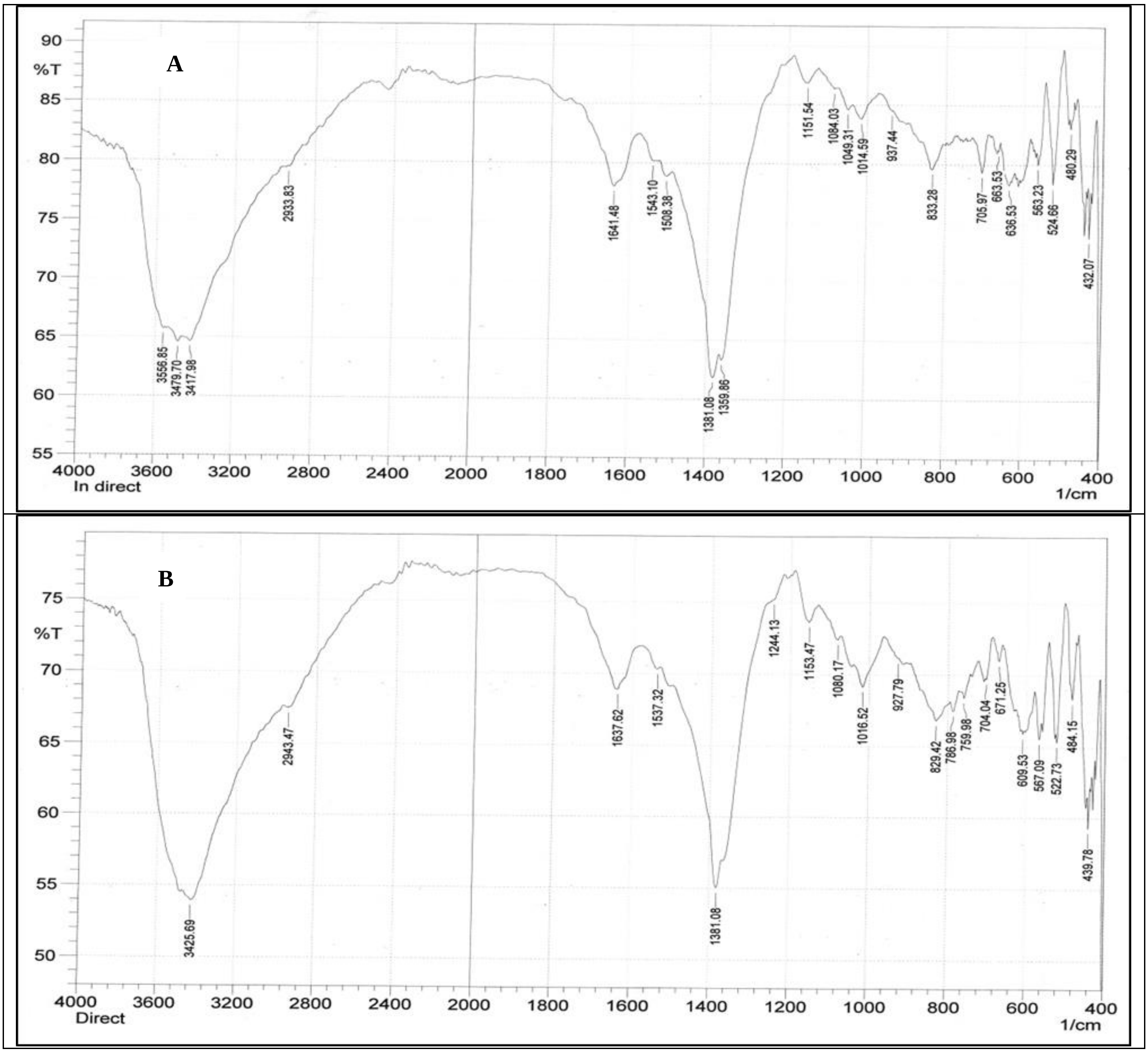

Fig. 4. FTIR Spectrafor AgNPs Nickel/Aluminum-LDHs (A) prepared by indirect Ion Exchange (B) prepare by direct Ion Exchange

Table 1. The peaks of the Ag PNs , Nickel/Aluminum-NO_3 -LDH, nanohybrid composite AgNPs Nickel/Aluminum-LDHs.

starch	AgNPs	LDH	Nano hybrid composite making ion exchange direct	Nano hybrid composite making ion exchange indirect	packet
3305	3305	3443	3425	3479	υ_{O-H}
1076	1085	1639.5	1637	1641	$_{O-H}$ δ
2930	2930		2942	2932	υ_{C-H}
1643	1643				$_{c-H}$ δ
1149 996,	1149996,		1154	1150	$_{C-H}\upsilon$cycle
	1450		1537	1543.2,1508.3	Ag-
	1335	1386.6	1380	1380	υ_{NO3}
		1083	1085		υ_{c-O-c}
		1017	1049		$\upsilon_{c-o- c}$
861		828	835		$_{C-H}\delta$cycle
	408.1	440	433		υ_{Ni-O}
	621.1	607	564		$_{Al-O}\upsilon$
726		704	705		$_{C-H}\delta$

C. X-ray diffraction (XRD)

The XRD spectrum for the prepared compounds, crystalline measures, milliliter coefficients (hkl), and crystal distances (d thickness layer value) at different angles (2θ) are shown using Bragg Law [31].

$2d\sin\theta = n\,\lambda$(1)

Where:

λ is Cu wavelength and equal to 1.540562 Å

n = 1, θ is different angle

Fig. 5 shows, the crystal levels of the silver nanoparticles are found at 2θ =38° withcrystalline level (111), and the thickness value of the layer (d)is 0.225 nm, also,at the crystal level (200) the thickness value of the layer is 0.2 nm at 2θ = 44°, while at the crystalline level (220) the value of the width layer is 0.143 nm at 2θ= 65°.The highest peak of prepared Ag nanoparticles is observed at the level (311) with d equal to 0.122 nm at 2θ= 78°, these results are in arrangement with the reported in reference [25].

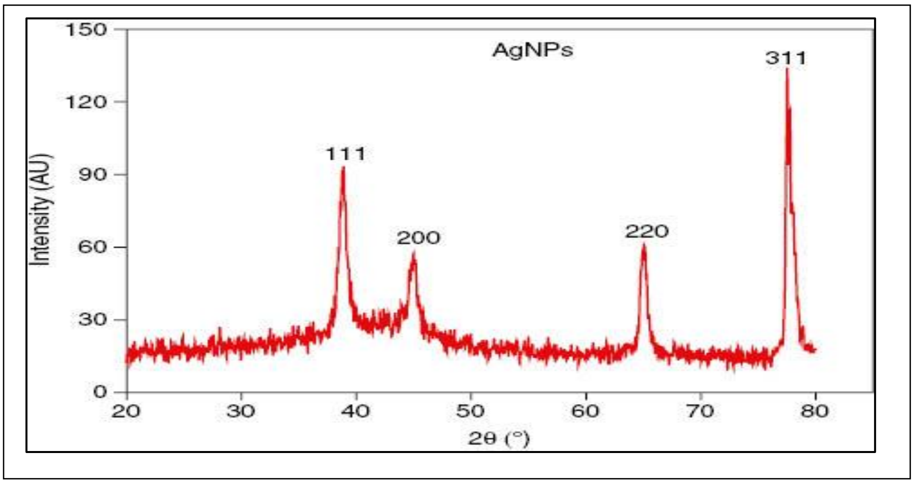

Fig. 5.XRD analysis of prepared Silver Nanoparticles.

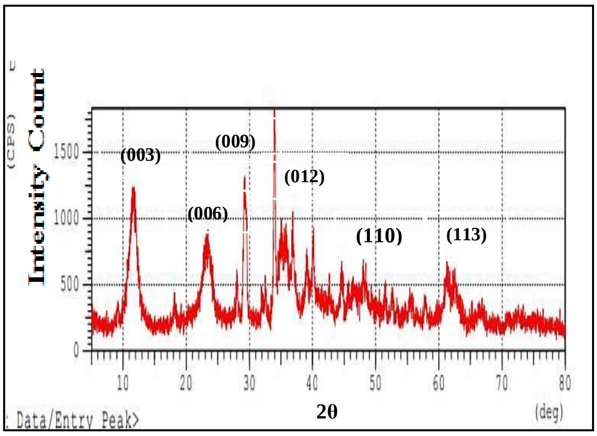

Fig. 6. XRD analysis of prepared Nickel/Aluminum-NO_3 –LDH.

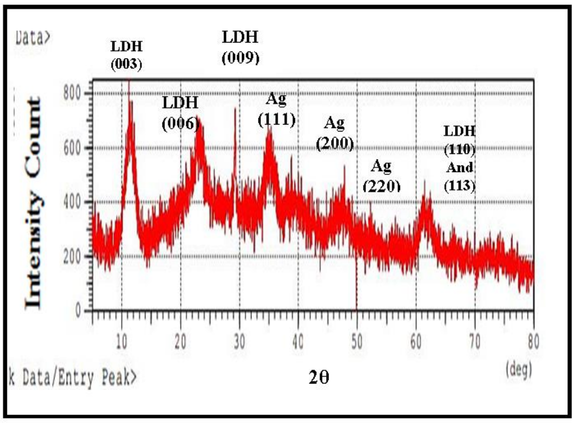

Fig. 7. XRD analysis for Nano Hybrid Compound that prepare by direct Ion Exchange.

Fig. 6 explicates that the XRD spectrum of the Nickel/Aluminum double hydroxide layers is observed at the crystalline level (003) with 2θ = 11.5º and the crystalline distance equal to 0.76 nm, also, the level (006) [27]

Appears at 2θ= 22º and with a distance of crystal equals 0.4 d nm, while the level (009) appears at 2θ= 29º and with a crystalline distance equal to 0.3 nm, while at 2θ ranged 37º-40º, it returns to the level (012) and with a crystalline distance equal to 0.25 nm, but the crystalline level (110) appears at 2θ=60º and 63º with the crystalline distance equals to 0.15 nm. At crystalline level (113) [32-33], theavarge crystal size is equal to 0.147 nm.

Fig. 7 demonstrates that the XRD spectrum of the hybrid nanocomposite Ag NPs-Nickel/Aluminum-LDHsthat were prepared using direct ion exchange method (the inventive method). It is observedin the success of inserting of Silver nanoparticles between the nickel/aluminum layers double hydroxide, where the crystalline level (003) appears at 2θ= 11.5º with a crystalline distance equal to 0.76nm, and the level (006) appears at 2θ=22º with a crystalline distance equal to 0.4 nm. Moreover, the level (009) occursat 2θ = 29º and with a crystalline distance equal to 0.3 nm.

The silver nanoparticles appear at the plane crystals (111) at 2θ =38ºwith the thickness value of the d = 0.225 nm, and the level (200) and(220) obtain at2 θ = 48ºand 68ºwith the crystal distance equal to 0.18 nm and 0.137 nm, respectively. In addition, two values of 2θ equal to 60ºand 63ºat (110) and (113)which avarge crystal size are owned the crystal distances equal to 0.15 nm and 0.147 nm,respectively

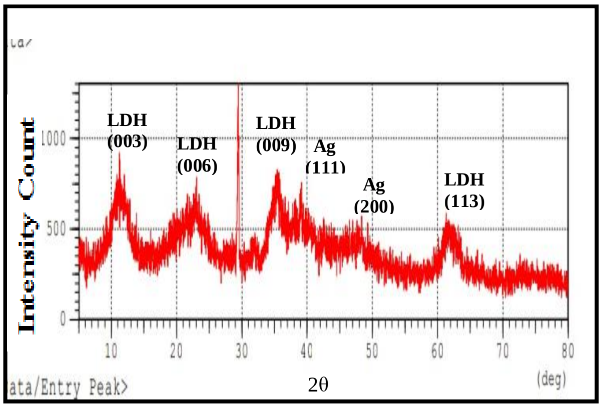

Fig. 8. XRD Nano Hybrid Composite prepared Ion Exchange Indirect.

Fig. 8 explains the X-ray diffraction spectrum of the hybrid nanocompositeAg NPs-Nickel/Aluminum-LDHs prepared using ion exchange indirect method as a common method, where it is observed in the same figure the appearance of the crystal levels of the layers double hydroxide and the silver nanoparticles as an indication of the success of intercalation of Silver nanoparticles between the Nickel/Aluminum layers double hydroxide, where the crystalline levels (003), (006) and (009) appear at 2θ = 11.5°, 22°, and 29° with the crystalline distances equal to 0.76 nm, 0.4 nm, and 0.3 nm, respectively, attitude to Nickel/Aluminum layers double hydroxide.

While silver nanoparticles appear at the crystalline levels in (111), (200) and (113) at 2θ = 38°, 48°, 63° and with the layer thickness value equal to 0.225nm, 0.18nm and 0.147nm, respectively. That is due to Nickel/Aluminum-LDHs.When the crystalline level (220) related to the nanoparticles, it did not appear, this shows that the innovative method is better than the common method for preparing the nanocomposite because it is more clearly in the appearance of the crystalline levels of the nanoparticle.The mean crystal sizes of prepared nanoparticles were detected using the Debye-Scherrer equation [34-37].

$$L = \frac{k \times \lambda}{\beta \times cos\theta} \quad (2)$$

Where, L is the mean crystal size in (nm), λ is the wavelength of the instrument source (Cu kα) in (nm), β is the full width at half maximum intensity in (mathematically transfer from degree to radian)[34] θ , is the Bragg diffraction angle and k is shaped constant (0.94) for spherical shape [35-36] and (0.9) for semi-sperical. The calculated mean crystal sizes $D_{(ave.)}$ are 18.709nm for Nickel/Aluminum-LDHs, 8.251 nm forAgNPs Nickel/Aluminum-LDHs that prepared by direct method, and 8.619 nmfor AgNPs Nickel/Aluminum-LDHs that prepared by Ion Exchange Indirect method. The average grain size per sample is calculated by calculating the grain size of all crystal surfaces and the results are exposed in Table 2.

Table 2. Calculatedmean crystal sizes for very prepared samplesusing Debye-Scherer equation for XRD data.

	Nickel/AluminumLDHs		
2Theta (deg)	FWHM (rad)	D (nm)	D_{ave} (nm)
34	0.3117	28.392	
29	0.433	19.833	18.709
11.787	1.053	7.903	
	AgNPsNickel/AluminumLDHs prepared by direct method		
11.56	1.71	4.908	
23	1.7	5.010	8.251
29	0.58	14.836	
	AgNPsNickel/AluminumLDHs prepared by indirect method		
29.46	0.195	8.619	
11.42	0.00		8.619
11.25	0.00		

D. Atomic Force Microscopy (AFM)

Figs. (9,11,13 and 15) demonstrate the surface characterized by Atomic Force Microscopy asthree-dimensional image and two-dimensional image to Ag nanoparticles, Nickel/Aluminum-NO_3–LDHs, and Nanohybrid composite AgNPsNickel/Aluminum-LDHs.

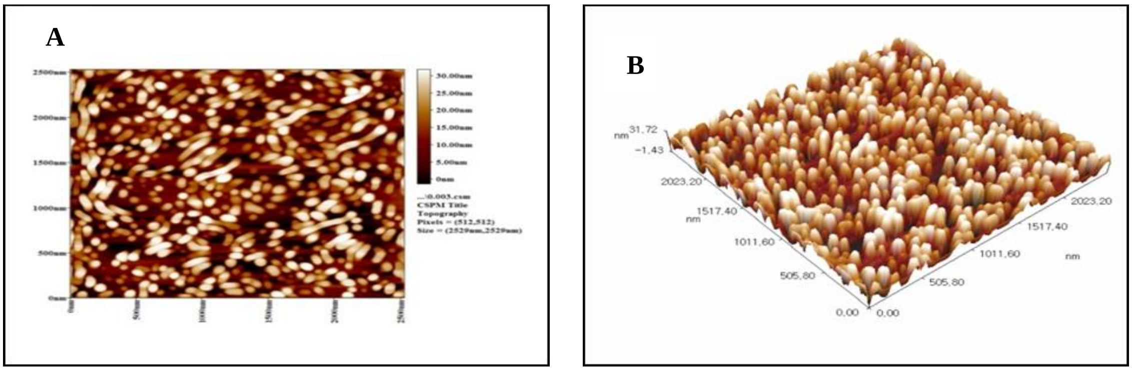

Fig. 9. Atomic Force Microscopy image of Ag NPs (A) two- dimensional image(B) three- dimensional image

Outside superficial for Ag NPs considered fig. 9A has exposed a two-dimensional image of the Ag NPs wherever particle bunches look though exposed fig.9B three-dimensional image takes surface for Ag NPs is agreed in height particle meetings which bound range (1.42 − 31.8) nm, that due to their polycrystals [38]. Fig. 10 displays the histogram for the average diameters (grain size) for Ag NPs is 74nm. The procedure of making Ag NPs get nanoparticles with diameters ranging between (40-100) nanometer, and maximum ratio for nanoparticles is 11.3% particles to the diameter 96nm,but the less percentage is 1.7% withparticles diameter equal to 40 nanometers. The outside surface of Nickel/Aluminum-NO_3–LDHs deliberate fig. 11A displayed a two-dimensional image of Nickel/Aluminum-NO_3–LDH, which tube-shaped particle seem although exposed fig. 11B as a three-dimensional image of surface Nickel/Aluminum-NO_3–LDH, which is agreed within elevation in particle bounds range from (0.21 nm to 13.5)nano meter.

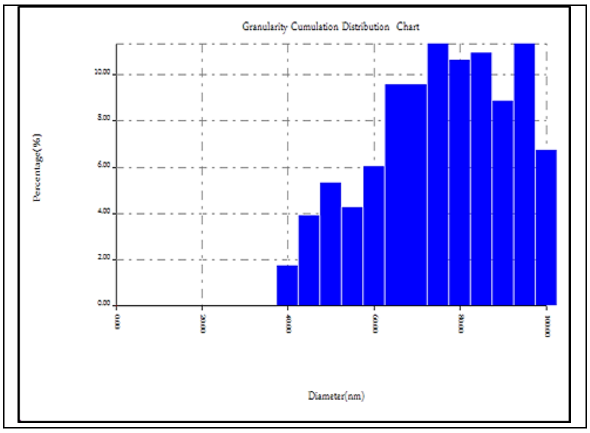

Fig. 10. Histogram of the ratios of diameter for Ag NPs.

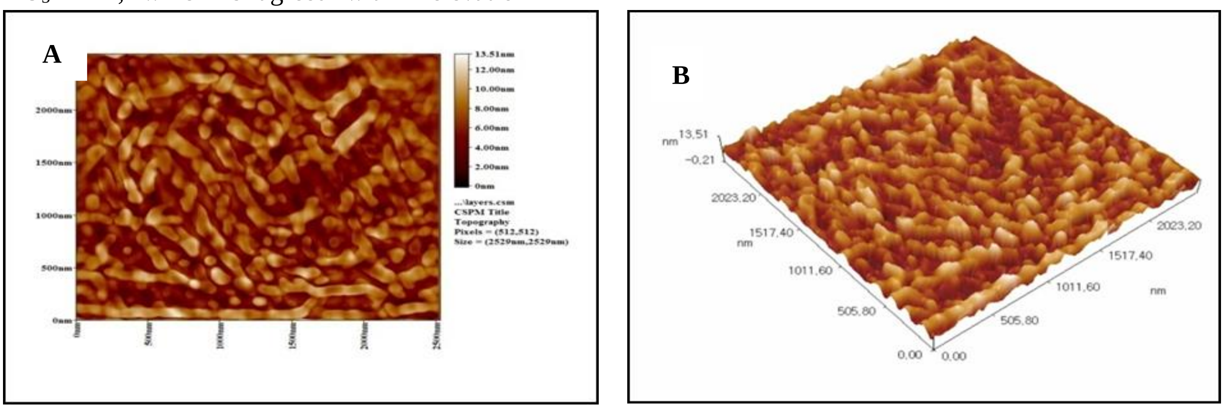

Fig. 11.Atomic Force Microscopy image of Nickel/Aluminum-LDHs (A) two- dimensional image(B) three-dimensional image.

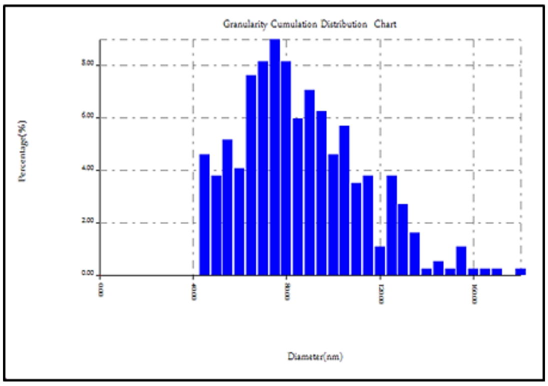

Fig. 12. Histogram of the ratios diameter for Nickel/Aluminum-LDHs

Fig. 12shows beforehand the intercalation procedure Ag PNswith Nickel/Aluminum-NO_3–LDHs, and the average grain size of Nickel/Aluminum-NO_3–LDH is 84nm. The range of diameters for Nickel/Aluminum-NO_3–LDHs to get nanoparticles lies between (45-180) nanometers and maximumratio for nanoparticles is 8.91% particles at the diameter of 75nanometres,but the low percentage is 0.271% at the diameter of the particles of 140nanometre.

Depended on the Ion Exchange indirect method, the shape of the outside surface of Ag NPs Nickel/Aluminum-LDHs prepared. Fig. 13A demonstrated a two-dimensional image for AgNPs Nickel/Aluminum-LDHs in where particle bunches' circular forms seem although exposed fig. 13B three-dimensional image for surface Ag PNs is agreed with the elevation of the particle bounds range from 0.09 nm to44.26 nm, which ensuresthe nanocomposite of Ag NPs with Nickel/Aluminum-LDHs is successfully preparewith rod –shape.

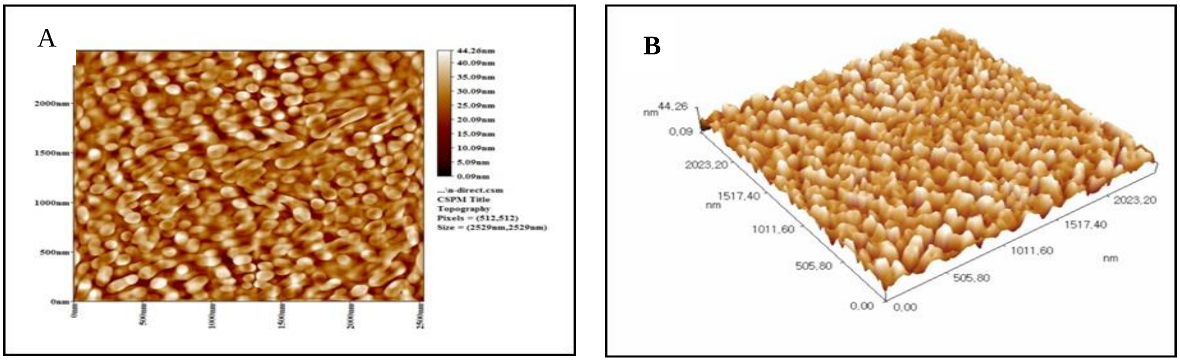

Fig. 13. Atomic Force Microscopy image of Ag NPsNickel/Aluminum-LDHs (A) two- dimensional image (B) three-dimensional image.

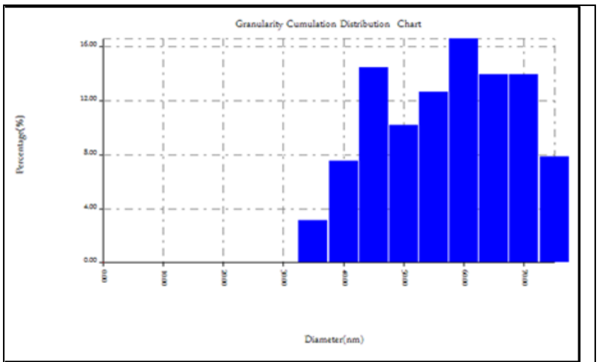

Fig. 14.Histogram of the ratios diameter nano hybrid composite Ag NPs Nickel/Aluminum-LDHs making by ion exchange indirect method

Fig. 14 displays the average diameters fornanohybrid composite Ag NPs Nickel/Aluminum-LDHs arranged that prepare by Ion Exchange indirect method and equal to 81nm.

The process of making Nickel/Aluminum-NO_3–LDH get nanoparticles in average diameters (particle size) is being among the ranged (60-150) nanometer. The maximum percentage for the prepared nanoparticles is 12.46 % particles with a diameter equal to 75nanometers, but the less percentage is 0.27% with the diameter of the particles equal to 150 nanometers. Based on the ion exchangedirect method, theoutside surface of AgNPsNickel/Aluminum-LDHs was prepared. Fig.15A and fig. 15B exposed two-dimensional image and three-dimensional image for Ag NPsNickel/Aluminum-LDHs with Ag NPs surface that gets a great particle with bounds range (0.01–46.39)nanometers, which successfully production as nanocomposite of Ag PNs with Nickel/Aluminum-LDHs with rod -shape.

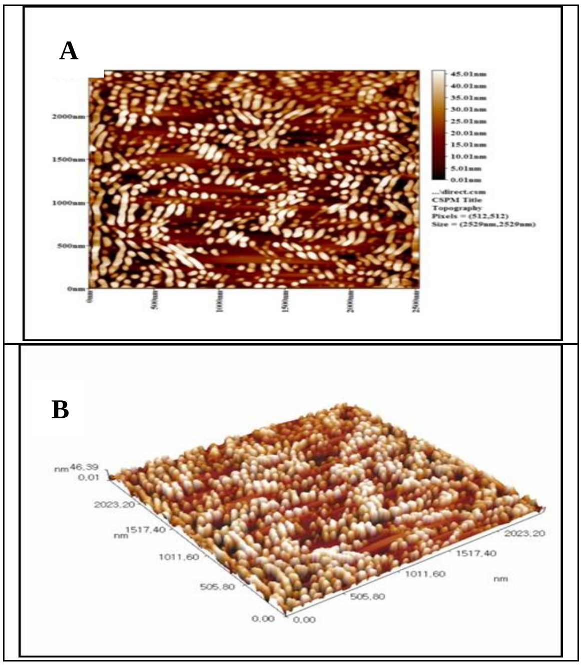

Fig. 15.Atomic Force Microscopy image of(Ag NPsNickel/Aluminum-LDHs)preprade by Ion Exchange direct method (A) two-dimension image (B) three-dimension image.

Fig. 16 and table 5 display the average diameters fornanohybrid composite Ag NPSNickel/Aluminum-LDHs making by ion exchange direct method is 55 nm. The prepared Nickel/Aluminum-NO_3–LDH has had nanoparticles diameters among (35-75) nm, the maximum percentage nanoparticles are reached to 16.56% with particles diameter 60 nanometers, but the lowest ratio is 3.13% with particle diameter equals to 35 nanometers with rod -shape.

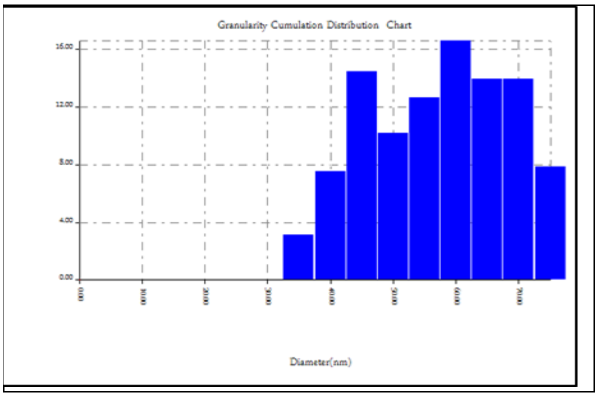

Fig. 16. Histogram of the ratios diameter for Ag NPsNickel/Aluminum-LDHs making by ion exchange direct method.

E.Antibacterial Performances of AgNPsNi/Al–LDH

The biocidal efficacy of the (Ag NPsNi/Al–LDH) was investigated in study by a colony-counting method for *Staphylococcus aureus* bacteria. layers double hydroxide did not show any antimicrobial activities,While Ag NPs and Ag NPsNi/Al–LDH manufactured by indirect ionic showed a low inhibitory effect [39-40].

The hybrid nanoparticle silver particles manufactured by direct ionic exchange method exhibited excellent activities against bacteria tested. In the tests, 100% of bacteria was killed after 3 h incubation in the PBS solution that containing the Ag NPsNi/Al–LDH manufactured using direct ionic exchange method, while, the death rate % is reached to 90 % in present (Ag NPsNi/Al–LDH) that prepared by indirect Ionic Exchange. On the other side, the death rate % reached to40% under using Ag NPs and0% for using the layers double hydroxide. Moreover, a good stability of our molecules to be tested for their inhibitory ability is attained as demonstrated by the 4th recycle runs (Figure 17).After Four runs, there was no apparent change in the kill activities against bacteria testedtahat mention in reference [40].

Ag NPsNi/Al–LDH exhibits their antibacterial potential through multifaceted mechanisms such as adhesion to bacterial cells, penetration inside the bacteria[41], free radical generation, and alteration of bacterial signal transduction pathways[42].

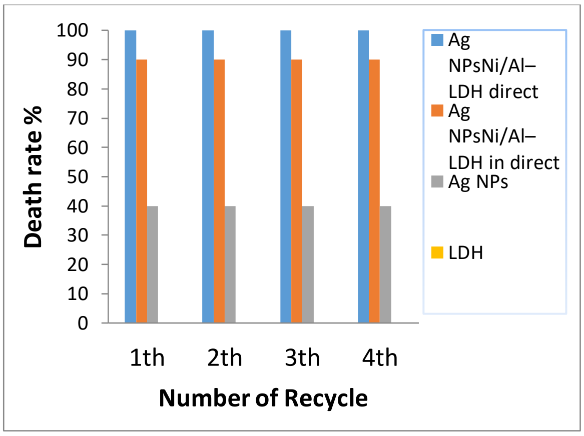

Fig. 17.The biocidal efficacy of the LDH, Ag NPs ,(Ag NPsNi/Al–LDH) manufactured by indirect and direct Ion Exchange method against *Staphylococcus aureus* bacteria.

F. **Growth Inhibition Study:** The growth behaviors of *S. aureus* in the presence of molecules to be tested weremeasured in the LB broth medium. Based on Figure 18, the growth of *Staphylococcus aureus* was monitored using the (O.D.600). The synthesized nanoparticles can inhibit the growth of bacteria that makes it suitable for repeated or long-term antibacterialapplications.A previous study has shown by Chen et al in 2011 [17] that AgNPs–LDH exhibited more toxic effects towards G-positive (*Staphylococcus aureus*) and G-negative*(Pseudomonas aeruginosa and Escherichia coli*) bacteria. Also the results confirmed that the *Escherichia coli*and the *Pseudomonas aeruginosa*displayed identical sensitivity at equaldoses of AgNPs.

Wypij*et al*in 2021 [43] found that the AgNPs–LDH have a good stability,protein capping agent and inhibited ATP synthesis in the bacteria cell as a potential antibacterial mechanism of action towards *S. aureus*,and other types of becteria such as*Klebsiellapneumoniae*, *Pseudomonas aeruginosa* , and *E.coli* .

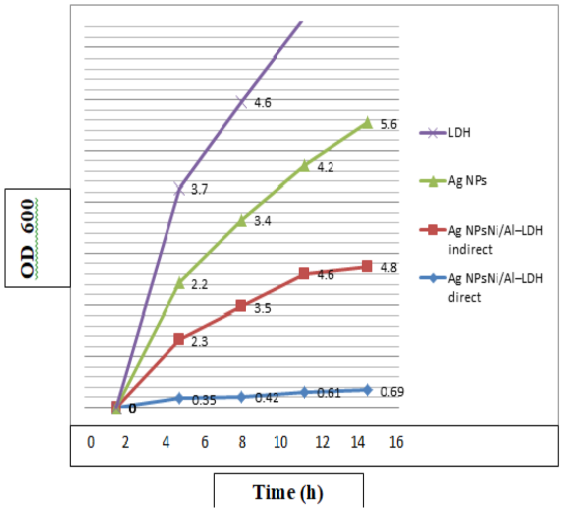

Fig. 18.Growth curves of *Staphylococcus aureus* after treated by the LDH, Ag NPs , (Ag NPsNi/Al–LDH)manufactured by Indirect and direct Ion Exchange method .

G.Antibiofilm activity of bacterial

Biofilm formation on a touching surfaces is initiated by bacterial adhesion, and the rate of adhesion significantly Differs based on the physical and chemical characteristics of the surfaces.Typically, the rate of bacterial adhesion on hydrophobic (plastic test tube) surfaces is higher than that on hydrophilic (glass test tube) surfaces[20-21].Therefore, in this study, the antibiofilm effect of the synthesized nanoparticles of bacteriawas tested on plastic test tube.Antibiofilm activity of the hybrid nanoparticle Ag NPs- Nickel/Aluminum-LDHs marked via the direct and indirect methods, Nickel / Aluminum layer double hydroxide and silver nanoparticles against Staphylococcus aureus bacteria were evaluated by measuring biofilm growth with crystal violet.The control without any particules added exhibited growth at an OD of 1.55. Ag NPsNi/Al–LDH -direct at concentrations of 50 µg/mL successfully reduced biofilm formation to 0.30 (Figure 19). Notable inhibition of colony growth would decrease OD in present Ag NPsNi/Al–LDH manufactured using an indirect ionic method and it was estimated 0.70.Ag NPs showed a low inhibitory effect hence, the OD is equal to 1.20. While the layers double hydroxide did not show any antibiofilmactivities. These results suggest that the both hybrid nanoparticle silver prepared in this work are able to prevent the bacterial infections by biofilm pathogenes in addition to killing the planktonic bacteria.

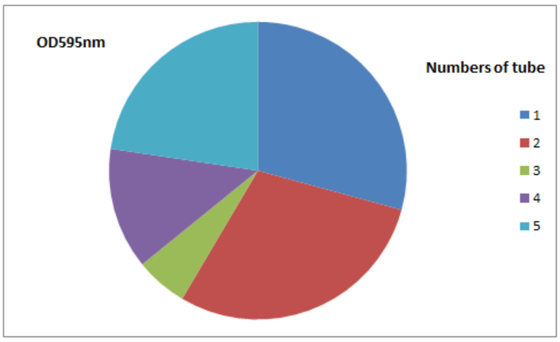

Fig.19. Antibiofilm activity of bacterial extracts on hydrophilic surface (test tube method). Tube 1: control without any particulesadded , Tube 2: Tube 3:added Ag NPsNi /Al–LDH manufactured by direct ionic method , Tube 4: added Ag NPsNi /Al–LDH manufactured by indirect ionic method , Tube 5: added Ag NPs against Staphylococcus aureus bacteria.

5. Conclusion

The production of hybrid nanocomposites using new ways was performed,that would reveal new physical and chemical properties of the produced molecule may prove efficient in solving several problems and in multiple fields. This study is making nanohybrid composite via intercalation AgPNs with layer double hydroxide form nanohybrid composite AgNPs Nickel/Aluminum-LDHs via Ion-Exchange method. Ag NPs produced were prepared via the reduction of Ag^+ using the starch solution. A produced superficial changed AgNPs condensed with layer double hydroxide via inclusion production Nickel/Aluminum-LDHs medium with Ag PNs. The present study, confirms that making nano hydride composite Ag NPsNickel/Aluminum-LDHs nanohybrid material having antibacterial activity, synthesizingAg NPs-Ni/Al-LDHwith controlled physico-chemical properties. It is one of the emerging endeavors that address present challenges in relation to safe use of Ag NPsNi/Al–LDH in therapeutics and drug delivery platforms. So this can be potentially applicable in food industries, cosmetic industries and has possibilities in the preparation of antibiotics and drugs against various pathogenic strains of bacteria. The resulte showed that the direct method of preparing nanopatical is the most efficient in inhibiting bacterial growth.

6. Conflicts of interest

"There are no conflicts to declare".

7. Acknowledgments

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