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ABSTRACT

Layered double hydroxides (LDHs) have been widely investigated in a wide range of applications in health, in the pharmaceutical industry and in the material of biotechnology industries. This material can be considered as a group of promising materials in the development of new health applications. The combination of phosphonic acid with LDHs material create a new hybrid material with new properties.In this work the synthesis of Zn/Al double layered hydroxides by chemical co-precipitation method (molar ration 2) and grafted with Diamino Dodecyl Phosphonic Acid (DDPA) via anion-exchange mechanism. The samples were characterized and confirmed by X-ray diffraction (XRD), and the presence of diamino dodecyl phosphonic acid (DDPA) was verified by Elemental analysis, BET analysis, and infrared spectroscopy. Both of the samples were found to be showing antibacterial activity. The zone diameters of Zn-Al- LDH were 40 mm and 25 mm for *Escherichia coli*(ATCC 25922) and *Streptococcus* (ATCC 25922)and 20 mm for *bacillus* (ATCC 25922) whereas the same for hybrid LDH (Zn-Al-DDPA) were43 mm , 32 mm and 25 mm for the same bacteria showing stronger antibacterial activity of the grafted material over the material itself. The experimental results confirm the application of Zn-Al-DDPA in the field of antibacterial activities and may offer a promising antibacterial elucidation to the society.

1. Introduction

Layered double hydroxides (LDHs) are one such potential inorganic matrices, containing sheets succession (Kadari et al., 2017) they are layered compounds by metal hydroxide sheet with positive charge and interlayer space filled with anion with negative electric charge. They can be expressed by the general formula $[M^{II}_{1-x}\ M^{III}_{x}\ (OH)_2]^{x+}[A^{n}_{x/n}\ .\ mH_2O]^{x-}$. (Cheng et al., 2019)in which M^{2+}(Mg^{2+} , Ca^{2+}, Zn^{2+}, Cu^{2+} , Co^{2+}, Ni^{2+}, etc.) and M^{3+} (Al^{3+}, Fe^{3+}, Ga^{3+}, Cr^{3+}) are divalent and trivalent cations and An^- (Cl^-, CO_3^{2-}, NO_3^-, etc)is an anion, thus having positive or negative charge layers (Marcato et al .,2013; Totaro et al., 2018)**.**Depending up on the nature of cations and their molar ratios M(II)/M(III), and so the anions type, many compounds, with vast difference, can be obtained. The range $0.2 \leq x \leq 0.33$ is generally acknowledged to be suitable for the synthesis of LDH compounds i.e. the ratio of M^{2+}/M^{3+} is in between 2:1 to 4:1 (Mishra et al.,2013; Zümreoglu et al., 2012)**.**These anionic clays, has been noticed to be of considerable uses due to their fascinating properties in compositional flexibility, anion and cation exchangeability, in many technologically in catalysis, separation technology, optics, medical science, low toxicity, superior biocompatibility, synthesis of films and coatings for anticorrosion, sensing and antibacterial applications (Ryu et al., 2010; Chen et al., 2011; Mei et al., 2018; Mishra et al., 2018).

ZnAl hydroxides are effective antibacterial agents for bacteria such as *Escherichia coli* and *Staphylococcusaureus* due to the hydroxides (–OH) and the nature of the metallic cations, where Zn^{2+} is one of the most active ones, due to its strong oligodynamic features (Ferraris et al.,2016;Gohi et al.,2019). Moreover, Zn ions possess excellent antibacterial ability and were widely used as inorganic antibacterial agents (Hue et al.,2013;wang et al.,2016). Also, many hybrid materials, organic hybrids, inorganic hybrids and organic-inorganic hydrids,have been tested and still have a lot ofl applications in biomedical science (Gohi et al.,2019).

.Wang et al.(2013) studied the antibacterial and the antimicrobial activity of Mg–Al–NO_3-

LDH with Ga^{3+}salicylidene-amino acid Schiff base complex intercalation. (Wang et al.,2013)

In previous works, we have prepared and characterized a new hybrid material (ZnAl- DDPA) by intercalating the double layered hydroxide synthesized in our laboratory (ZnAl-LDH) prepared by coprecipitation method using diamino dodecyl phosphonic acid (DDPA). In this work, *Streptococcus*, *Bacillus* (gram negative) and *E. Coli* (gram positive) antibacterial activity has been studied using a synthesized LDH and its hybrid material.

2. Experimental

2.1. Materials

The reagents used in this study were Zn Al- Cl and ZnAl-DDPA synthesized in the laboratory (Villemin et al.,2010;Kadari et al., 2017), the raw materials used were $ZnCl_2$ (Riedel-de Haën), $AlCl_3$.6H2O (Panreac) NaOH(99%, Riedel De Haen).

The bacterial stains used in this work are *Streptococcus* (ATCC 25922 gram negative) and *Bacillus* (ATCC 25922 gram negative) , *E. Coli* (ATCC25922 gram positive).

2.2. Methods

2.2.1. ZnAl-Cl Synthesis

The ZnAl-HDL with a molar ratio of 2 :1 was synthesized by the co-precipitation method. About 150 ml of ZnCl2.7H2O (0,66M) et de AlCl3.6H2O(0,33M) are added to an reactor initially containing 100 ml of distilled water at 25 °C under stirring. The pH of the reaction was maintained constant at 10.5±0.1 with the addition of 1.5 M NaOH solution under vigorous stirring. Duration of the reaction was 24 h at the ambient temperature (25°C). The precipitate obtained was washed and centrifuged several times and was dried at room temperature then crushed.

2.2.2. Synthesis of ZnAl-DDPA

The intercalation of the diamino dodecyl phosphonic acid (DDPA) was made by the method of exchange of ions by the direct reaction between the DDPA and ZnAl-Cl (Feng et al.,2006) . The hybrid material was obtained by mixing the DDPA with ZnAl-LDH. Briefly, 1g of DDPA dispersion (1.736 × 10-3 M) is dissolved in 50 ml of water and mixed with 20 g for LDH [ZnAl-Cl] (Ratio 1/20). The reaction mixture is left under stirring for 24 hours. It is filtered and dried for 48 hours at the ambient temperature.

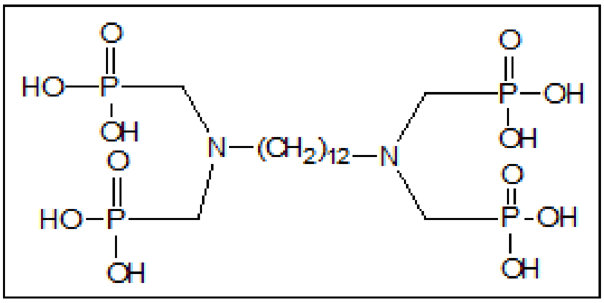


Figure 1: diamino dodecylphosphoni acid Molecule.

2.3. Characterization techniques

2.3.1. XRD Analyses

The X-ray diffraction (XRD) analysis was performed on a PAN analytical X'PERT Prodiffractometer with CuKa radiation((1.5405 A°) and with an operating voltage of 40 kv and a sweeping of 2ufrom 5 to 100.

2.3.2. FTIR Analyses

Fourier-transform infrared (FTIR) spectra of the samples were obtained with a Perkin–Elmer

Spectrum 16PCequipped with a thermostat.

2.3.3. BET analyses

Specific surfaces areas and pore size were analyzed by the Brunauer Emmett Teller (BET) method. Measurements were made by adsorption and thermal desorption of N2 at 77°K and the results were collected by Quantachrome Autosorb-6.

2.3.4. Elementary analyses

Elementary analysis was carried out using a Shimadzu ICPS-7500 inductivly coupled plasma (ICP) atomic emission spectroscopy.

2.4. Antibacterial activity

ZnAl-Cl and Zn-Al-DDPA antibacterial activity was examined against three test organisms, namely *E. coli* (ATCC 25922), *Streptococcus* (ATCC 25922) and *Bacillus* (ATCC 25922), by qualitative method (Mishra et al.,2017)to evaluate the potency of each sample. 0,5 ml of cultures were diluted in 20 ml of Muller Hinton liquid, this last was melted by heating and then cooled before placing in contact with the bacterial suspension. One milligram of each sample was placed at well-marked places on bacterial culture. The mixture was deposited in Petri dishes of 90 mm in the form of wells.

The inhibition zones of each sample around spots at regular intervals after 24h incubation, at 37 °C, were noticed visually.

3. Results and discussion

3.1. X-ray diffraction

The XRD patterns of the hybrid (ZnAl-DDPA) and ZnAl-LDH are presented in the figure 2 ,The basal

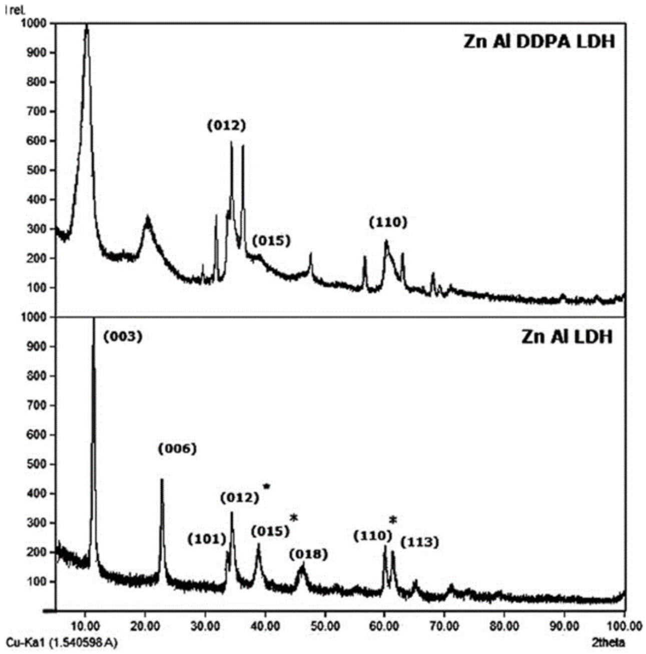


Figure 2: XRD patterns of Zn/Al-LDH and Zn/Al-DDPA.

plane spacing of the pristine LDH (Zn/Al-LDH) is 7.55 A° and an inter metallic distance of 1.53 A°. With a successful intercalation there is an increase in the basal plane of ZnAl-DDPA caused by the intercalation of DDPA, herein the spots can be indexed to a rhombohedral symmetry (space group,R -3m) (Mandal et al.,2009)andwere indexed in an hexagonal structure. The (a) and (c) lattice parameters were calculated using (110) and (003) reflections, respectively.

3.2. Fourier transformed infrared spectroscopy analysis (FTIR)

Bands of Fourier transformed infrared spectroscopy (FITR) are shown in Figure3, the band 3458 cm^{-1} corresponds to the stretching vibration of the hydroxide groups of the molecules water, The peaks located at 1120 and 1020 cm^{-1} are attributed to the bending vibration of adsorbed phosphate P=O, P-O respectively (Villemin et al.,2010), showing that the pristine LDH grafted by phosphonic acid (DDPA) and the secondly significant difference between the two spectra is that there are two peaks of low intensity recorded at 2980 and 2857 cm^{-1} which due respectively to the elongations of -CH_2 asymmetrical and symmetrical.

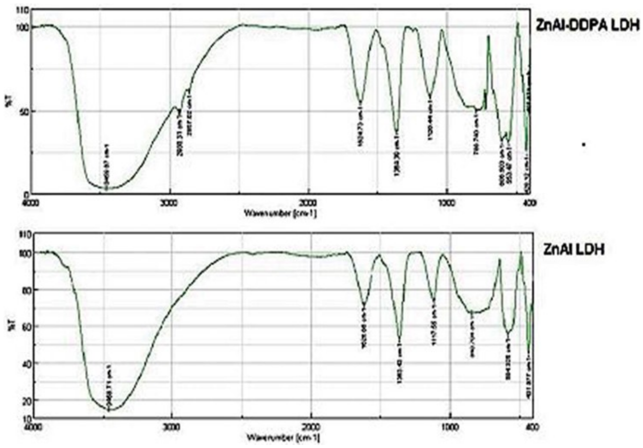


Figure 3: FTIR spectra of ZnAl-LDH and ZnAl-DDPA.

3.3. Elemental analysis and BET analysis

The molar ratio of the synthesized LDH materials was determined with use of AAS elementary analysis, and the results are given in Table 1, it can be distinguished that there are deviations between initial atomic ratios in solution and final atomic ratios were observed. It is observed from the data that some amount of Zn is being replaced by addition of DDPA to the system, and it is remarkable that the absence of Cl^- in the composition of LDH hybrid (ZnAL-DDPA) proves the exchange of ions between the chloride ions and the DDPA.

Specific surface areas of ZnAl-LDH and ZnAl-DDPA were estimated and presented in **Table 2**. It is evident from the table that the specific surface area (BET) decreased after DDPA intercalation in ZnAl-LDH.

3.4. Antibacterial Tests

The inhibition zones are shown in Figure 4 and the diameters of their sizes are summarized in Figure 5 and Table 3. The experiments were done three times to confirm the obtained results. The results revealed that ZnAl-Cl material sample has excellent antibacterial activity against the mentioned bacteria; presumably for the fact that ZnAl LDH presents a positive charge. This might be as a result of peptide polyglycogene existing in the cell border of gram positive bacteria and operating with plenty of pores that allowed stronger molecules to penetrate into the cell easily (Mohamed et al.,2013). Another elucidation is zinc ion, it has a magnificent effect on both bacteria (Sugarman., 1983) indicating that metallic ion-exchanged LDH diffused in water

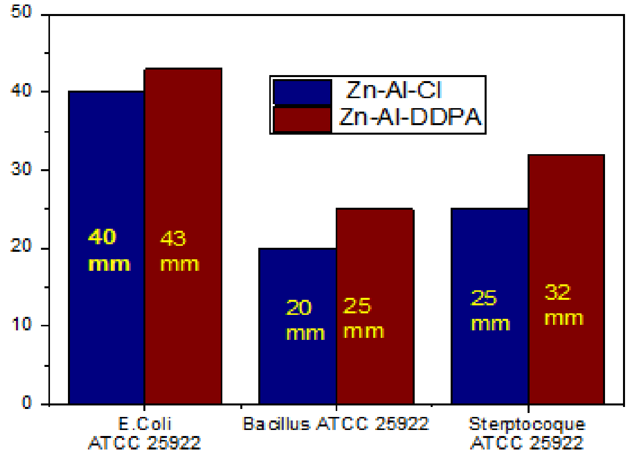


Figure 5: Histogram representing the zone of inhibition of bacterial.

attracted bacteria by electrostatic forces, which explain the perfect antibacterial activities (Dizman et al.,2007; Parolo et al.,2010).

4. Conclusion

Zn-Al LDH an Zn-Al-DDPA synthesized in the laboratory and characterized by XRD, FTIR, BET, and the

Sample composition	Zn (wt%)	Al (wt %)	Cl (wt%)	P (wt%)	C (wt%)	Zn^{2+}/Al^{3+} molar ratio
ZnAl-LDH	44.16	9.08	11.98	11.98	–	2.0
ZnAl-DDPA	15.48	3.29	-	-	6.01	1.9

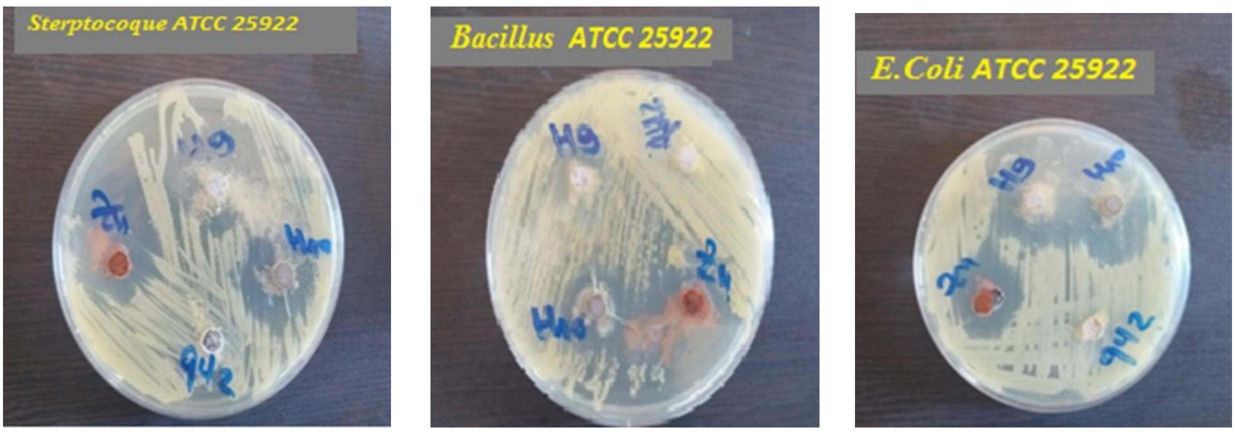
Table 1: Chemical composition of ZnAl-LDH and ZnAl-DDPA.

	BETsurface area(m2g-1)	Pore volume(cm3g-1)	Average pore size(nm)
ZnAl-LDH	62.4854	0.2908	18.6157
ZnAl-DDPA	10.3297	0.009791	3.7912

Table 2: Pore properties and specification surface area of ZnAl-HDL and ZnAl-DDPA.

	Bacterial strains		
	Gram-negative	**Gram-positive**	
	E.Coli ATCC 25922	*Bacillus* ATCC 25922	*Sterptocoque* ATCC25922
ZnAl-Cl	40mm+0,5	20mm+0,7	25mm+0,1
ZnAl-DDPA	43mm+0,05	25mm+0,08	32mm+0,04

Table 4: Antibacterial test results of adsorbents materials ZnAl-LDH and ZnAl-DDPA against bacterial stains.



H9 :ZnAl-LDH .H10 :ZnAL-DDPA
Figure 4: Inhibition zone tests of adsorbents materials ZnAl-LDH, ZnAL-DDPA against bacterial stains

elementary analysis showed high antibacterial activity against all the tested microorganisms such as *E.coli* (ATCC 25922), *Streptococcus* (ATCC 25922*), Bacillus* (ATCC 25922). The antibacterial activity was evaluated with respect to the inhibition zone size in mm, therefore, this hybrid material exhibited very good antibacterial potency, and such a result gives evidence of the feasibility of the samples as "active antibacterial" materials.

Acknowledgements

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