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Ciprofloxacin Intercalated with ZnO to Produce a Nanohybrid Used as a Delivery Machine

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Abstract
Ciprofloxacin (Cip) with zinc layered hydroxide (ZLH) as the precursors nanohybrid (CipN) was synthesized under an aqueous environment. The synthesis, bioactivity and the anti-bacterial activity of CipN nanoparticles has been evaluated against a wide variety of bacterial strains such as staphylococcus, streptococci, E. coli and hellcobacter. The anti-bacterial activity of CipN has a highly safe compound and may be considered for combination therapy against many bacterial strains, due to its potential synergistic effect with important antibiotics. Powder X-ray diffraction showed that the basal spacing of the nanohybrid was 2.5nm, resulting in the spatial orientation of Cip molecules between the interlayers of ZnO oriented with the direction of the z-axis. SEM images indicate the confirmations of the success of the intercalations. The AFE (Atomic Forcing Electrons) shows the average size of particles found was 86nm. The FTIR study showed that the intercalated CipN spectral feature is generally similar to that of the Cip free molecule, but with bands slightly shifted .This indicates that some chemical bonding of Cip presence between the nanohybrid interlayers was slightly changed due to the formation of host–guest interaction. The nanohybrid is of mesopores type with 54.5% drug loading and enhanced thermal stability. The drug CipN nanohybrid was found to be sustained and therefore has good potential to be used as a nanohybrid drug which is more effective than the free one. The vitro bioassay study showed that the CipN has a mild effect on the hepatocyte cells, more than its counterpart, free Cip.
Keywords: ZnO; nano materials; antimicrobial activity; intercalations.

1- Introduction

Systemic drug delivery leads to distribution of the drug throughout the body through blood circulation, which can lead to drug concentration accumulation in unwanted parts of the body which causes severe side effects. Additionally, conventional drug administration methods do not provide satisfactory pharmacokinetic profiles because the drug concentration rapidly falls below the desired levels [1].

Chemotherapy is the treatment of bacteria cells with an antibiotic drug. The main property of Chemotherapy acts by the inhibitions of bacteria cells as cells in the digestive tract, producing side effects. To overcome these side effects, researchers are now searching for efficient and safe transport carriers which prolong the exposure time to drugs and target bacteria cells without targeting healthy cells. In the past few decades, many carriers have been developed and generally can be classified into four major groups: viral carriers, recombinant proteins, organic cationic compounds and inorganic nanoparticles [5, 6]. Recently, inorganic nanoparticles have attracted considerable attention due to their versatile features such as wide availability, good biocompatibility, rich surface functionality, potential capability of target delivery and controlled release of the drug from the inorganic nanoparticles[7–9]. Calcium phosphate, gold, carbon nanotubes, silicon oxide, iron oxide, layered double hydroxides and Zinc Oxide (ZnO) are examples of inorganic nanoparticles, which have been intensively investigated in different studies by different groups [10–15]. Among these are their ease of laboratory preparation with a controlled particle size [16]. The drugs can be easily loaded into the interlayer space by ion exchange, comparing with other nanoparticles, which need further modification such as surface functional. LH, also known as hydrotalcite-like compounds are formed by layered units in which metal cations are octahe-drally coordinated with hydroxyl groups, as in the brucite ($Mg(OH)_2$) structure. Zinc layered hydroxide (ZLH) is a compound whose structure is derived from brucite. One quarter of the octahedral coordinated zinc cations are displaced from the main layer to tetrahedral sites located above and below each empty octahedron and can be represented by the general formula $M^{+}(OH)_{2-x}(A^{m-})_{x/m}\,nH_2O$ where M^{+2} is the Zn^{2+} and A^{m-} is counter ions with (m -) charge[21]. Because of the ZLH anionic exchange capacity, many active compounds can be intercalated into ZLH interlayer cations by trivalent cations which leaves a residual positive charge that is stabilized by inter layer anions. These include the anti-carcinogenic agent gallic acid[22], linoleic acid[23], sunscreen materials such as 2-amino benzoic acid and 4-amino benzoic acid [24], nucleoside monophosphate, DNA [25] and pharmaceutical, cosmeceutical, and nutraceutical compounds[26] .Various methods have been adopted for preparation of ZLH and its nanohybrids, namely hydrolysis of salt and oxides[27], urea hydrolysis[28], precipitation with alkaline solutions [29] and solid state reaction [30]. The direct reaction of zinc oxide (ZnO) is simple and easily used either for aqueous or nonaqueous systems. Such a method is economic and environmentally friendly as fewer steps and chemicals are involved. Cip is an antibiotic agent, which is a family of drugs much used for

bacterial treatment. Intense research has been conducted to investigate the effect of Cip on the bacteria cell as well as its delivery [31–34]. Lately, many articles have described the preparation of ZLH as a starting material followed by intercalation of the anion. However, to the best of our knowledge, little work has been published on the use of ZnO as a starting material to intercalate drug activity [35, 36]. Therefore, the main objective of this work was to explore the potential use of ZnO as a starting material for the intercalation of Cip for the formation of a new CipN nanohybrid . The resulting nanohybrid was then used as a controlled release formulation of drug activity of Cip. A cytotoxicity study of nanohybrids was also carried out.

2- Experimental:

2-1- Synthesis of Materials

All chemicals used in this experiment were obtained from various chemical suppliers and used without any further purification. All solutions were prepared using deionized water. CipN was synthesized by ion exchange method using ZnO as a starting material. About 1g of ZnO was firstly suspended into 100 mL of deionized water with stirring for 15 minutes. Ciprofloxacin solution of concentrations; 100mg/ml was prepared by dissolving a respective amount in 50 mL H_2O and the volume was adjusted using a volumetric flask with deionized water. The solution of the Cip was added drop wise into ZnO suspension with constant stirring. The pH of the solution was adjusted to 8 with 0.5 M NaOH aqueous solution to obtain a white precipitate. The slurry solution was vigorously stirred on a magnetic stirrer for 2 hours and the aging process was further continued in a bath shaker for 18 hours at 60 ^oC. The obtained product was centrifuged, thoroughly washed with deionized water to wash away any contaminants and then dried in an oven at 70 ^oC. The obtained material was then powdered for further use and characterization.

2-2- Characterization

Powder X-ray diffraction patterns of nanohybrids were obtained at $2–40^0$ on a Shimadzu diffractometer, XRD-6000 using CuKα radiation (λ =1.5418 Å) and a dwell time of 4 degrees per minute.

2-3- Kinetic Release

Recently, nanotechnology-based drug delivery systems have emerged as powerful methods not only to enhance drug efficiency but also to minimize side effects of cancer chemotherapy. The release of Cip from the nanohybrid host into different media were accomplished using distilled water at pHs =4, 6.5 and 8 by adding 10mg of the nanohybrid into 100 mL of the solution. The pH solution in distilled water was obtained by adjusting its pH using HCl or NaOH and pH values were measured using a pH meter. Taking 3.5ml of the solutions at time interval filtered and measuring the accumulated concentration by cicel Uv-Visible spectromphotometer. The cumulative release pattern was fitted to first, pseudo-second order kinetics models. The second pseudo order was more fitted with release, the accumulations of the Cip was 80% of the sample studied.

3- Results and Discussion: (Morphology of nanocomposites)

3-1- Powder X-ray Diffraction Analysis

The PXRD patterns of the unbound chemical ZnO and CipN nanolayered structure are shown in Fig. 1 and Fig. 2 respectively. The Powder X-ray diffraction pattern of ZnO reflects five sharp peaks between 30 – 40^o region, corresponding to reflections of 100, 002, 101, 102 and 110 lattice planes which indicate high crystallinity and this represents a distinctive pattern of the metal oxide. While the PXRD pattern of the counterpart anion CipN Fig. 2 shows some reflection peaks and demonstrates crystalline nature of this chemical. Reflection peaks diffracted at lower 2θ angle with an increase in the basal d spacing is evidence of inclusion of the anion Cip inside the interlayer spacing of ZLH. The resulting nanolayered material shows the presence of ZnO phase, which indicate an incomplete reaction. The expansion basal spacing of nanohybrid material synthesized has a 2.0 nm interlayer distance which can be indexed to the (003), (006) and (009) planes corresponding to the Ciprofloxacin indicating successful intercalation of the organic anion between the inorganic ZLH interlayer has taken place. Using the full width at half maximum of the main peak (003) for the CipN nanocomposites in to the Debye–Scherrer equation [see Eq. (1)], the average particle sizes were calculated as 40nm.

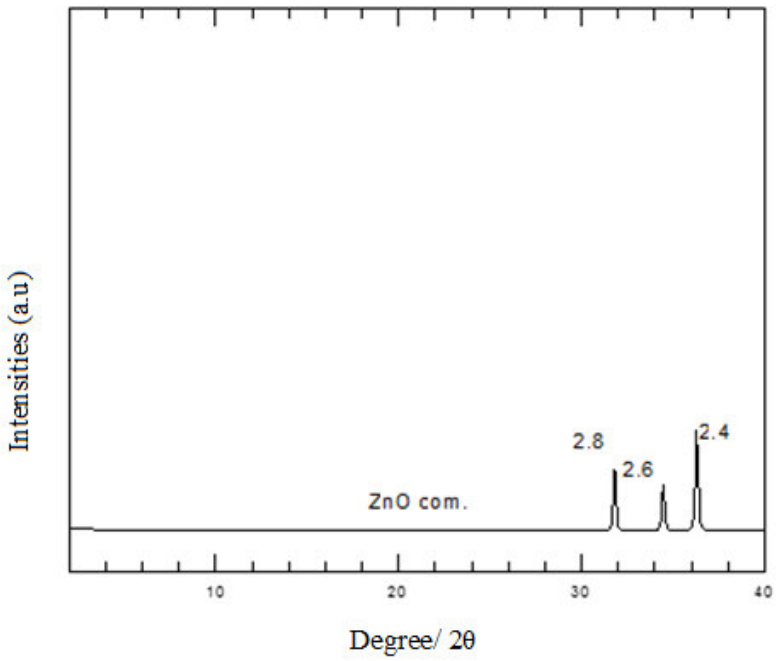


Figure 1 represents the PXRD of the ZnO pure shows the corresponding planes.

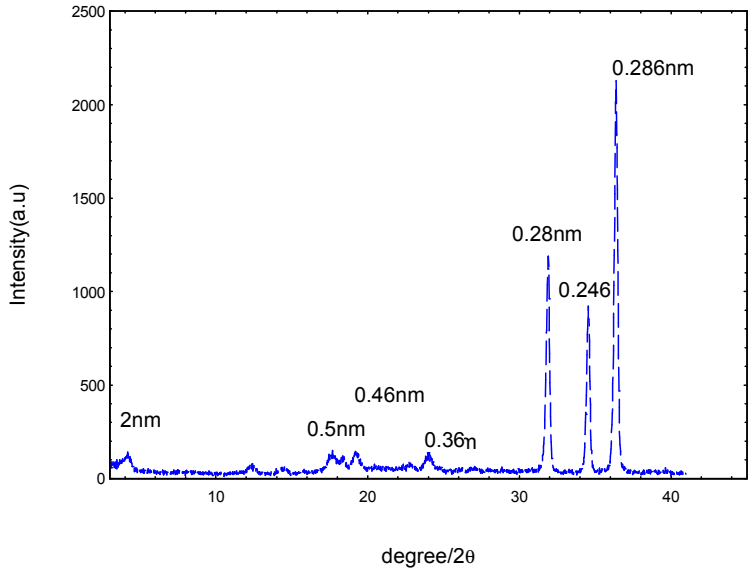


Figure 2 represents the PXRD of the CipN shows the corresponding planes.

D=0:9λ / βcosθ Eq.(1)
D is the average crystalline particle size of the ordered (crystalline) domains, λ is the wavelength of the X-ray (0.15418nm), θ is the diffraction angle in 2 θ /degree and θ in radian is the measured full width at half maximum intensity. The particle size was expected to increase after intercalations of the CipN with the ZLH and the drug however, it was observed that the particle size of the CipN nanocomposites decreased. This might be explained by the pro-longed and vigorous stirring at a high speed after the addition of the Cip and ZnO, and this is in accordance with a similar result reported [38].

3-2- Scanning electron microscopy (SEM)
Figure (3) show the Scanning electron microscopy (SEM) electron micrographs of the ZLH and CipN nanocomposites at a magnication of 10000, which revealed very strong agglomeration due to the vander Waals force between the particles. The degree of agglomeration decreases after the intercalations of the nanoparticle with the Cip and ZnO to obtain the CipN nanocomposites [see Fig (3) and (4)]. Figure (5) shows the average size

distribution for the MNP nanoparticles obtained by dynamic light Scattering (DLS). The average size was found to be 86nm which is in accordance with the results obtained from the XRD [40].

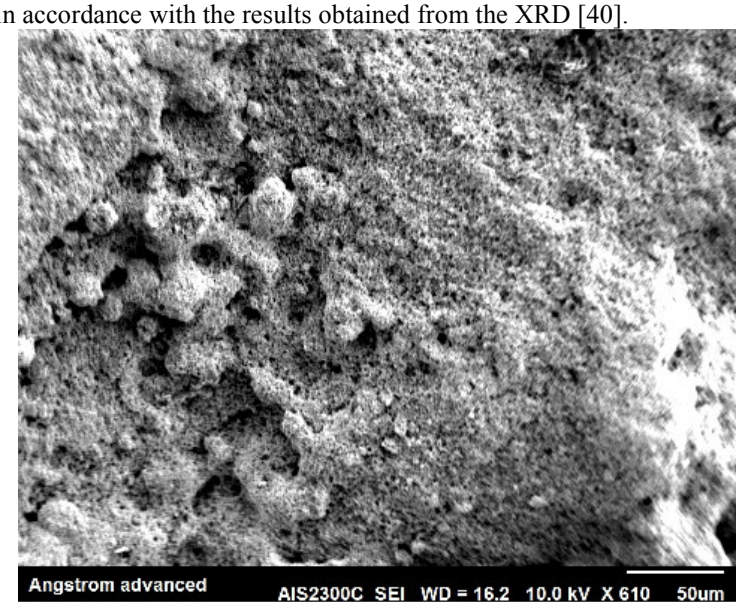


Figure 3 SEM scanning electronic microscope of the CipN nano particles shows high agglomerations.

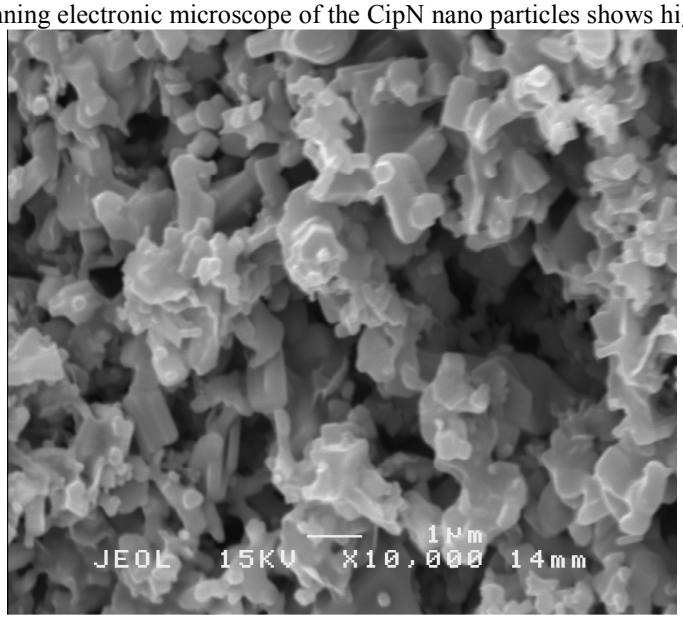


Fig (4) SEM image of pure ZnO shows the hexagonal crystals.

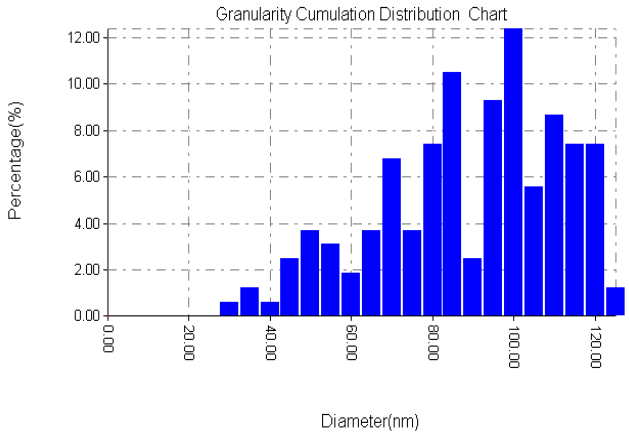


Figure 5 shows the distributions of particles size with an average of 86nm.

3-3- Atomic Force Electron (AFM):

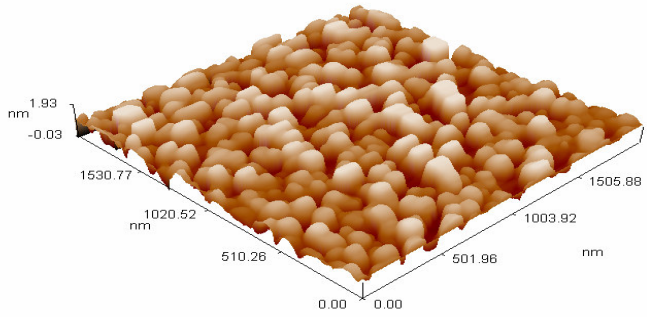


Figure 6 shows the three dimensional particles with d distance of 1.93 by the Atomic Force Electron (AFM). The distance in the directions of Z axis is with accordance of XRD.

3-4- FTIR technique:

The FTIR spectrum of the hybrid is a complement of the XRD results and provide further evidence for the intercalation. In addition, some absorption bands are slightly shifted due to the interaction of Cip, Fig (7) shows the host layer. The typical broad absorptions bands of zinc layer hydroxide. In Fig.(8) the spectrum of the nano composite shows the superposition of OH groups with the water molecules at 3531 cm−1 observed and the band due to the (C–O-H), the stretching vibration of C-H stretching in the organic chain at 2825 cm^{-1}, the asymmetric and symmetric stretching of C=O appears at 1622 cm−1. The bands at 1708, 1610 and 1280 cm−1 attributed to characteristic of the vibrations of 5FC present in the nanohybrids that is indicating the presence of Cip molecules in the nanohybrids. In the ciprofloxacin Fig (8) shows the bands located at 1492 and 1458 cm−1 due to the stretching vibration of C-N in the aromatic ring. The presence of carboxylate group, COO can be deduced by the observation of bands at 1691 are due to C=O stretching, the band at 1573 are due to C-O-H bending, the bands at 777 and 800 cm−1 are symmetric and anti-symmetric vibrations of the COO- group in the Cip intercalated in the inter layers of ZnO Fig(9). Whereas a band at around 1012, 1062 cm-1 is corresponded to(–C-0-) stretching vibration, the results of FTIR give the evidences of the successions of the intercalations of the Cip in between the layers of ZnO.

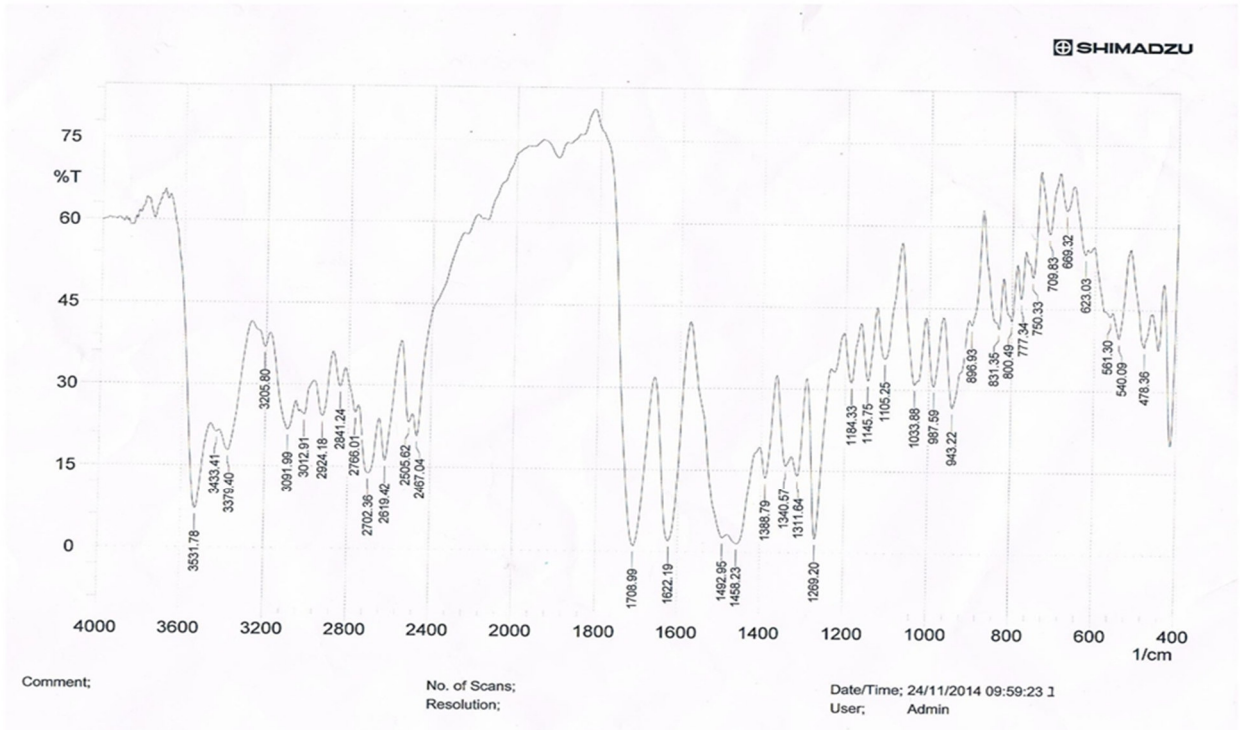
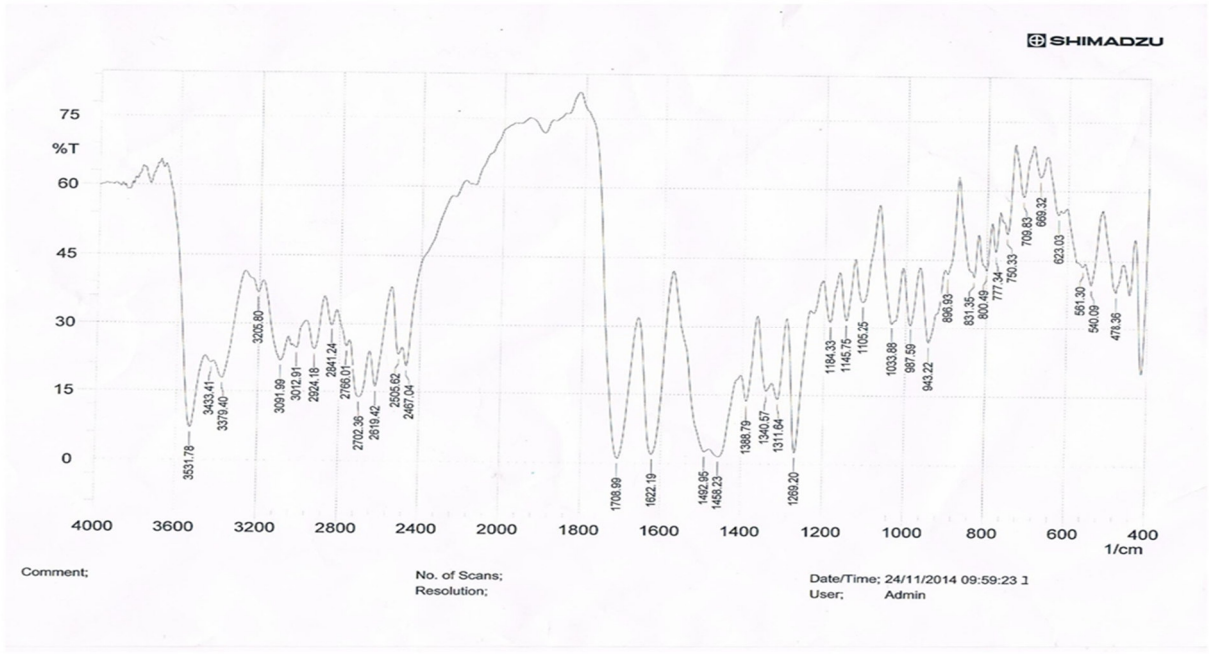


Fig (7) FTIR of the free Cip. Layer shows the characteristic peak of the drug.



. Fig (8) FTIR spectrum of the CipN nano composite shows the Cip characteristic harmonies.

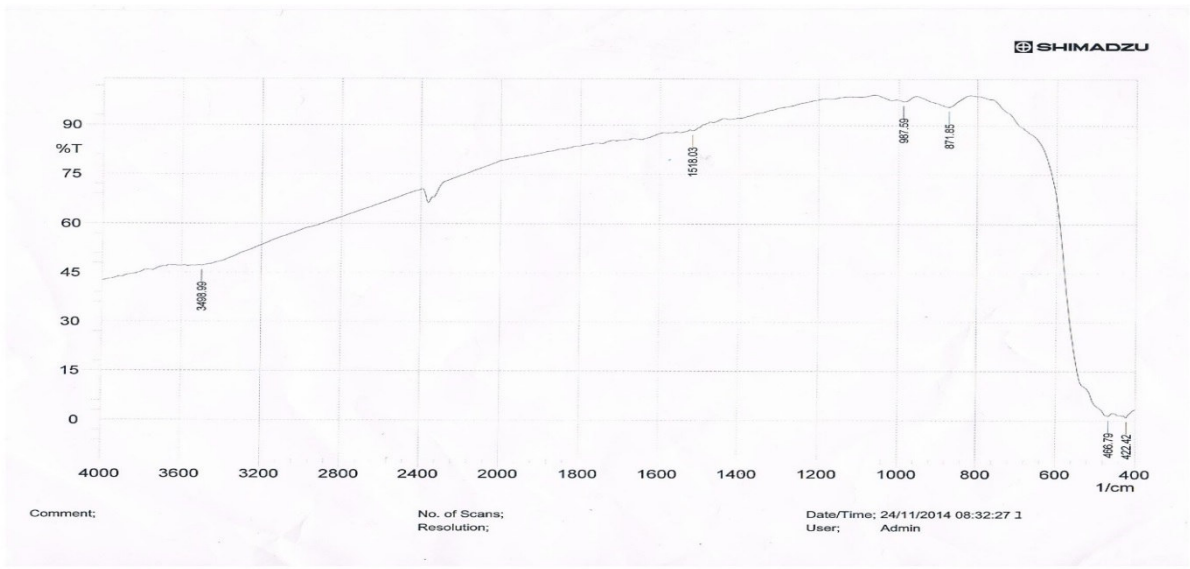


Fig (9) FTIR of ZnO shows the functional groups found in the structure of the zinc oxide non intercalated.

4- Anti-bacteria applications:

Ciprofloxacin is used to treat a wide variety of infections, including infections of bones and joints, endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, and chancroid.[37].

Ciprofloxacin only treats bacterial infections; it does not treat viral infections such as the common cold. Although for certain uses including acute sinusitis lower respiratory tract infections and uncomplicated gonorrhea, ciprofloxacin is not considered a first-line agent.

Ciprofloxacin occupies an important role in treatment guidelines issued by major medical societies for the treatment of serious infections, especially those likely to be caused by Gram-negative bacteria, including *Pseudomonas aeruginosa*. Ciprofloxacin in combination.

In case of gram positive bacteria, CipN exhibited highest antibacterial activity against

Streptococcus with an inhibition zone (51mm) at 10mg/ml concentration, whereas minimal activity was shown against *Helicobacterium* with inhibition zone (33mm) at 10mg/ml concentration.

Gram positive bacteria Ciprofloxacin exhibited highest antibacterial activity against *Streptococcus* with an

inhibition zone (50mm) at 10mg/ml concentration, whereas minimal activity was shown against Helicobacterium with inhibition zone (44mm) at 10mg/ml concentration.
Gram positive bacteria CipN exhibited highest antibacterial activity against *Staphylococcus* with an inhibition zone (22mm) at 0.05mg/ml concentration, whereas minimal activity was shown *against Helicobacterium* with inhibition zone (15mm) at 10mg/ml concentration.
Gram positive bacteria Ciprofloxacin exhibited highest antibacterial activity against *staphylococcus* with an inhibition zone (33mm) at 0.05mg/ml concentration, whereas minimal activity was shown against *Helicobacterium* with inhibition zone (15mm) at 0.05mg/ml concentration, Table (1).

Table 1: Inhibition zone diameter in mm in the indicated concentration

								Type Of Bacteria
10	5	1	0.5	0.25	0.1	0.05	**Concent-ration Mg/ml**	
49,49,50	47,48,45	40,45,42	44,43,39	41,42,42	34,34,35	29,27,29	***Cip***	***E.coli***
47,46,48	43,45,43	38,38,39	38.39,40	32,33,34	23,27,19	16,19,17	***Cip+Zn***	
46,50,50	40,40,38	34,36,34	40,33,35	36,35,37	28,29,29	29,30,33	***Cip***	***Staph***
43,39,38	36,36,37	37,38,35	36,35,35	34,34,33	21,20,20	18,22,20	**Cip+Zn**	
50,50,50	52,50,49	39,40,40	36,38,37	36,35,37	27,28,28	25,24,25	***Cip***	***Strep***
49,47,51	42,39,40	46,38,39	36,37,37	34,35,35	18,17,17	15,13,15	***Cip+Zn***	
44,46,46	38,40,42	34,34,34	32,30,34	31,30,28	22,23,	15,16,21	***Cip***	***Helico***
33,34,36	34,30,29	27,27, 28	23,24,24	23,21,24	19,20,18	18,15,16	***Cip+Zn***	

5- Statistical Analysis:

Statistical analysis for the result show there is significant differences between the free Cip and the intercalated Cip with zinc oxide against different bacteria.
Statistical analysis included factorial experiences analysis 4 × 2 x 7 with 3 replicates. The factors analyzed are the type of bacteria, type of compounds and the concentration of compounds. 0.05 is the level of probability that was used to identify a significant difference. The significant differences between the averages were also tested by using the test less significant difference (LSD) at the level of probability of 0.05 [41].

Table (2): Inhibition zone (mm) of CipN and Cip free against four types of bacteria

Compounds	Concentration (mg/ml)	Bacteria				Mean of compounds	$LSD_{0.05}$ Compounds
		E. coli	*Staph. aureus*	*Strep.*	*Helico*		
Cip-ZnO	**0.05**	28.3± 0.88	30.6± 1.15	24.7± 0.66	17.3±0.88	**30.96 B**	**0.846**
	0.1	34.3± 2.3	28.6± 0.33	27.6± 0.33	22.5± 0.55		
	0.25	41.7± 0.33	36± 0.33	36± 0.33	29.6± 0.88		
	0.5	42± 0.33	36± 0.33	37± 0.33	32± 0.33		
	1	42.3± 0.57	34.7± 0.88	39.6± 0.88	34± 0.33		
	5	46.6± 0.57	39.3± 0.66	50± 0.88	40± 1.52		
	10	49.3± 0.33	48.7± 0.33	50.3± 0.57	45.3± 0.88		
Cip-Free	**0.05**	17.3± 0.66	20± 1.2	14.3± 0.33	16.3± 1.85	**36.72 A**	
	0.1	23± 0.33	20.3± 0.33	17.3± 0.33	19± 0.28		
	0.25	33± 0.33	33.6± 0.57	34.7± 0.57	22.7± 0.88		
	0.5	39± 0.57	35.3± 2.08	36.6± 0.57	23.6± 1.15		
	1	38.3± 0.33	36.7± 0.66	37.6± 0.33	27.3± 0.0		
	5	43.6± 1.52	36.3± 2.6	40.3± 0.88	31± 1.15		
	10	47± 1.45	40± 1.33	48± 0.0	34.3± 0.66		
Mean of Bacteria		**37.57a**	**34.26c**	**35.31b**	**28.22 d**	**$LSD_{0.05}$ Interference 2.394**	
$LSD_{0.05}$ Bacteria		**0.639**					

* The numbers refer to mean ± Standard error.
*Various vertically capital letters indicate significant differences (P<0.05) between the compounds.
*Various Horizontally small letters indicate significant differences (P<0.05) between bacteria.

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