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Structural Charecterization and Controlled Release Analysis of 5-Fluorocytosine-ZnO-LH Nanocomposite Against Candida Albicans

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Abstract: Znic Layered hydroxides (Zn\LH) have recently fascinated researchers due to their wide application in various fields. These inorganic nanoparticles, with excellent features as nanocarriers in drug delivery systems, due to its significant properties which can be increased drug loading, reduced side effect of the drug, have the potential to play an important role in healthcare. Owing to their outstanding ion-exchange capacity, many organic pharmaceutical drugs have been intercalated into the interlayer galleries of LHs and, consequently, novel nanodrugs or smart drugs may revolutionize in the treatment of diseases. Layered hydroxides, as green nanoreservoirs with sustained drug release and cell targeting properties hold great promise of improving health and prolonging life. In this study we have synthesized nanohybrid of 5-FC with Zinc \ LHs nanoparticls via sol-gel method , the structure of NPs have been characterized using XRD, FT-IR, and AFM. 5-Flucytosine is well known as powerful oral antifungal drug in the treatment of OPC in human which caused by Candida albicans. Candida albicans isolated have been used to determine the cytotoxicity of 5-FC NP by treatment of oral fungi isolated with intercalated compound. The entire drug loading Nanoparticles show enhanced ability to inhibit fungal cells proliferation, where, we saw that highest inhibition zone at 25 mg/L concentration and MIC at 5 mg/L concentration.

Keywords: Drug Delivery, Inorganic Nanoparticles, Layered Zinc Hydroxed 5-Flucytocine, Candida Albicans.

I. INTRODUCTION

Nanotechnology is the ability to observe, measure, manipulate and manufacture things at the nanometer scale, A nanometer (nm) is one-billionth of a meter, That's very small(1).Nanotechnology is employed to describe materials, devices and systems with structures and components exhibiting new and significantly improved physical, chemical and biological properties. Nanotechnology is almost a household word now-a-days, or at least some word with ‘‘nano’’ in it, such as nanoscale nanoparticle, nanomaterials, nanophase, nanocrystal(2). Nanomaterials such as two-dimensional (2D) nanosheets have recently gained much attention due to their unique physical and chemical properties. Excellent intercalation properties of 2D layered material offer a new scope for developing hybrid materials at nanoscale dimensions or the so-called nanocomposite. This type of material offers a variety of applications in industries and the environment such as anion-exchanger(3) catalysis, delamination, as well as in medical science, and more(4). Covers a broad range of topics and encompasses a variety of subjects such as chemistry, physics, materials science, engineering, biology, and medicine (5). In a word, nanotechnology deals with nanoscale materials which are also called nanomaterial. Nanomaterias (nanocrystalline materials) are materials possessing grain sizes on the order of a billionth of a meter; It is used to describe materials with one or more components that have at least one dimension in the range of 1 to 100 nm (6). Nanomaterials have large properties of surface atome, and the surface of any material is where reactions happen. Because of nanoparticals huge surface area and thus very high surface activity, nanotechnologists can potentially use much less material, The amount of surface area also allows a fast reaction with less time, Therefore, many properties can be altered at the nanoscale. That's the power of nanotechnology(7). ZnO, LH are attracting much attention in drug delivery and gene therapy because of their biocompatibility, anion-exchange property, nontoxicityit is still urgent to expand the kinds of drugs that can combine with Zinc LHs to form the safe and efficient drug delivery systems More importantly, the mechanisms of the drug–LH interactions, drug controlled release and delivery efficiency .

A. Layered Hydroxides as Drug Delivery Systems

Layered hydroxides salt (LH), a layered inorganic compound is gaining attention in a wide range of applications, particularly due to its unique anion exchange properties. Recently, Zinc layered hydroxide have gained considerable attention due to their unique features as

nanocarriers in drug delivery systems, Their anion exchange property allows loading of various drugs into the interlayer lamellae of LH, which leads to modification of the charge density of the internal and external surfaces, resulting in greater chemical stability, cell targeting function, and high surface area The rate-controlled drug delivery property resulted in the reduction of drug concentration fluctuations and maintains drug concentration at the desired level for longer periods of time, decreases side effects, and reduces the number of doses and therapy duration, which leads to more effective treatment

B. 5-Fluorocytocine (5-FC)

5-fluorocytosine (5-FC) is an oral antifungal agent (8). It was synthesized in 1957, as a potential anti-tumour agent (9), but it was not sufficiently effective against tumours (10). Four years later, 5-FC proved to be active in experimental candidosis (11) and, in 1968, it was used to treat human candidosis (12).5-Fluorocytosine (5-FC), a fluorinated pyrimidine analog as figure (1), is a synthetic antimycotic prodrug that is converted by cytosine deaminase to 5-fluorouraci (13).

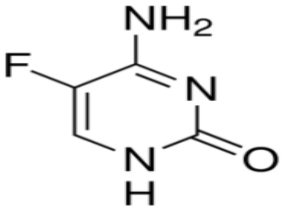

Figure1. Structure of 5-fluorocytosine (4-amino 5-fluro-1, 2-dihydropyrimidin).

5-FC itself has no antifungal activity, its antimycotic activity results from rapid conversion of 5-FC into 5-FU (Figure 2) which inhibit fungal RNA and DNA synthesis (14). 5-FU, on the other hand, cannot be used as an antimycotic drug, since it is highly toxic to mammalian cells and also because it is only poorly taken up by fungal cells (15).

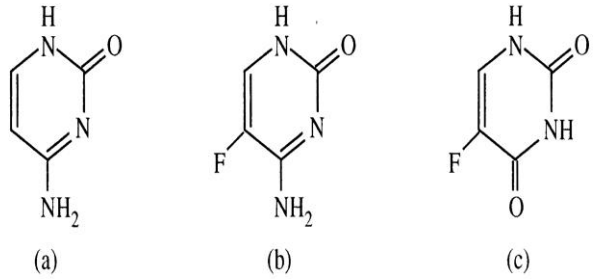

Figure2. Chemical structures of (a) cytosine, (b) fluorocytosine and (c) 5-fluorouracil.

5-FC is most active against yeasts, including Candida albicans. This is the species responsible for the majority of cases of OPC (Oropharyngeal candidiasis) in human (16). Objective of this work as following: (1) To synthesis and characterize Zinc oxide Layer Hydroxide Nanoparticles. (2) To incorporate 5-Fluorocytosine into ZnO Layered hydroxide nanoparticles. (3) To estimate the toxicity of 5-FC-Zno-LHs nanocomposite on Candida albicans. This work is to build up a charge-neutral, highly pharmaceutically active drug intercalated Zinc-LH in an attempt to gain a novel therapeutic delivery system.

II. MATERIALS AND METHOD

A. Materials and devices

Devices: Sensitive Balance, Centrifuge, oven, Micropipettes, Fourier transform infrared (FT-IR) Spectrophotometer, UV-Visible Spectrophotomete, Atomic Force Microscope (AFM) , Autoclave, Incubator, Laminar flow cabinet, pH-meter, Shaker Water bath.

B. Chemical materials

5-fluorocytocine antifungal (98.0%) purchased from Avra science company (India-Hayderabad), Zinc oxide (99.0%) purchased from Merk (Germany), HCl (Analytical Rasayan), Na_2CO_3 and NaOH (Fluca), Nutrient broth (Himedia-India) and DMSO (Fluca).

C. Method

1. Preparation of 5- fluorocytocine \ ZLH Nanohybrid

ZLH nanoparticls will be prepare via Sol- gel method from ZnO and 5-fluorocytosine, The ZLH nanohybrid will prepare by the direct reaction of ZnO (host) with the 5-fluorocytosine (guest anions). All solutions will prepare using deionized water, 100 ml solution of 5-FC (1.2 gm) will prepare. This solution will mix with the solution prepare of ZnO (1 gm) in 50 ml de-ionized water, in conical flask, with magnetic stirrer at 37 C °for 24 Hour, formation of gel suspension will start, aging at 40C °for 18 Hour, cool, centrifug at 5000 Rpm for 20 min, and wash for four times with de-ionized water, dry in oven at 40C°, grinding and keep for further use and characterization.

2. Determination of Calibration Curve to 5-FC

Calibration curve is the relation between concentration of 5-FC solution and it is absorption it at λ_{max} of 5-FC. Dissolved a known weight 0.002 g. Of 5-FC in 20 ml of the de-ionized water, prepare four Sequential concentration within the range (5-20 ppm) from 5-FC solution. Have been measured of absorption for these concentration at lambda maximum (λ_{max}) 305nm for 5- FC, Then draw of standard curve between absorption and concentration, then calculated according to an already obtained a linear relationship.

3. Study of 5-Flurocytosine into Aqueous Solutions:

The release of 5-Flurocytosine drug from the ZLH nanohybrid into the media will accomplish using various aqueous solutions,

solution No. (1): Sodium Carbonate Solution Na_2CO_3 (0.5 M) will prepare by dissolved 10.6 gm of Na_2CO_3 in 200 ml. of de-ionized water.

solution No. (2): Sodium Hydroxide Solution NaOH (2 M) will Prepare by dissolved 4 gm. of sodium hydroxide in 100 ml. of deionized water. With PH= 13 specific by PH meter.

Solution No. (3): Hydrochloric acid Solution, HCL (2 M) will prepare by mixing drops of HCL with 95 ml. of

deionized water until PH equal2, then complement of volum to 100 ml.

I will add 10 mg. of prepared nanocomposite on each one of previous solutions with shaker water bath, then caleculate absorbance with time by UN-vis spectroscopy.

4. Characterization of 5-FC-zinc /LHs nanohybride:

The characterization study of nanohybrid composites by Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction Spectroscopy (XRD), Atomic Force Microscopy (AFM).

5.Test of antifungal activity of 5-FC-LZH nanohybride:

It will test inhibition activity of 5-FC-Zno-LHs Nano-composite against Candida albicans yeast ,then measure of inhibition zone.

6. Test of antifungal activity of 5-FC as free drug:

It will test inhibition activity of 5-FC as free drug against Candida albicans yeast, then measure of inhibition zone.

III. RESULTS AND DISCUSSION

A. Powder X-ray Diffraction

Powder X-ray diffraction patterns of the solids obtained that indicate after hydrothermal treatment at ZnO-LH nanohybrids have been formed (Fig.3). For sample ZnO-LH at, the basal reflections are recorded at 1.42nm (003), in this case That the parameter c= 3 (d 003) =4.3 nm approximately, the diffractions of d= 0.71nm and 0.36nm is for the planes (006), and (009), respectively. On the other hand, the basal reflections of sample ZnO (fig.4) are recorded at 0.281, 0.259 and 0.247 nm respectively. The evidence for phase structure of the as-prepared sample was obtained by XRD pattern, as shown in (Fig. 4) All the diffraction peaks can be indexed to those of hexagonal ZnO. After refinement, the lattice constants, a=3.251 Å, c=5.210 Å, were obtained, which is very close to the reported value for ZnO (a=3.253 Å, c=5.209, JCPDS card, No.80-0075). Powder XRD patterns of the 5FC-ZnO-LH samples showed that the full width of half maximum (FWHM) value of (003)=0.43 deg., which indicated an increase of the crystallite size with 5FC-ZnO-LH increase. And all the sharp diffraction peaks indicate the good crystals of the obtained nanostructures.

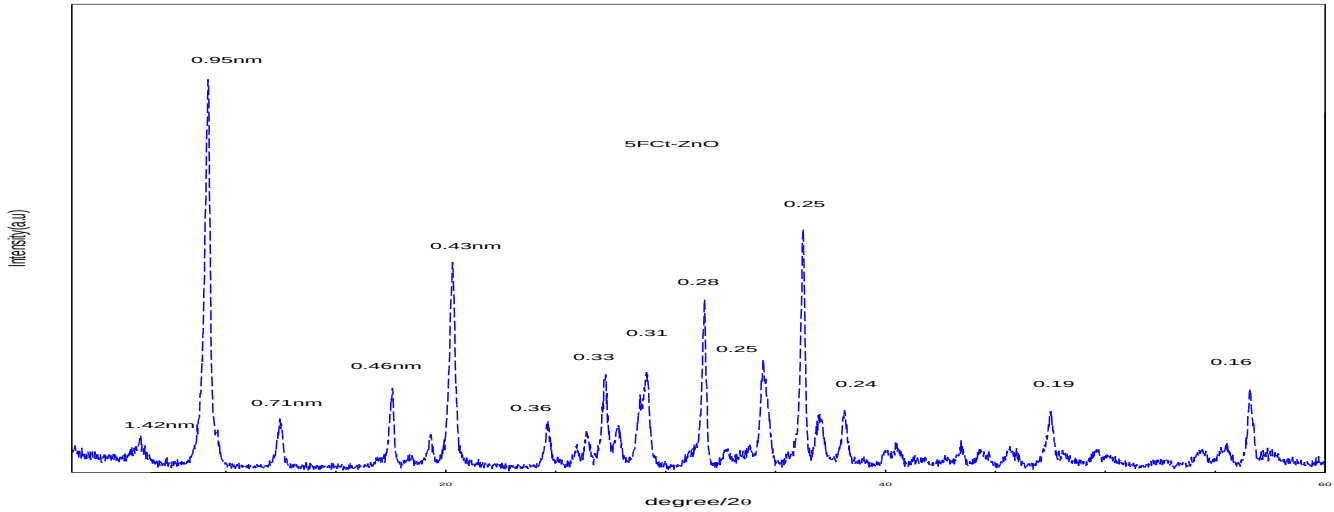

Fig3. Powder XR Diffractions of 5FC-ZnO-LH shows the evaluations of (003)plane diffractions at d=1.42nm.

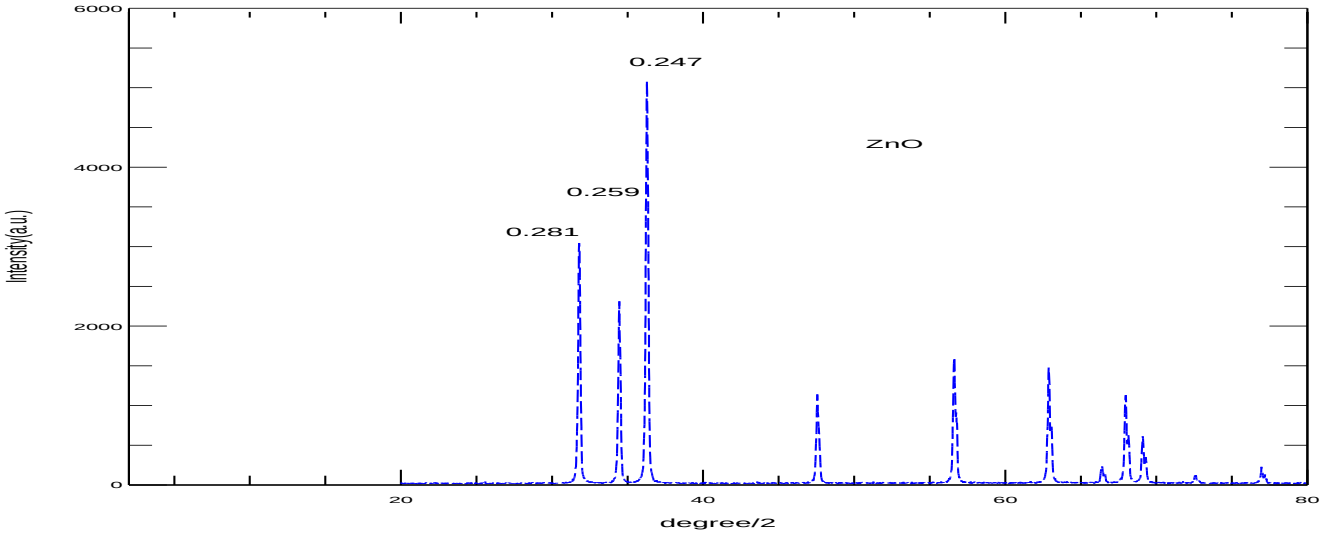

Fig11. XR Diffractions of ZnO shows the characteristic planes diffractions of (101). (011) and (002).

B. The 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 both the anion (Fig.5) and the host layer (Fig.6). The typical broad absorptions bands of zinc layer hydroxide superposing with the anion hydroxide observed and the water molecules at 3524 cm^{-1} and the band due to the (C–O-H) stretching vibration of C-H stretching in the organic chain at 2825 cm^{-1}, the asymmetric and symmetric stretching of C=Oappears at 1691 cm^{-1}. The bands at 1643 , 1610 and 1280 cm^{-1} attributed to characterstic of the vibrations of 5FC present in the nanohybrids that is indicating the presence of 5FC molecule in the nanohybrids. In the 5FC-Zinc nanocomposites(fig.7) shows a combination spectrum of both the host ZnO and the guest anions, the bands located at 1384 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 , 783 and the band 893 cm^{-1} are symmetric and anti symmetric vibrations of the COO- group in the 5FC intercalated in the inter layers of ZnO **(17)**. Which are attributed to asymmetric and symmetric vibration, respectively **(18), (19)** whereas a band at around 1012, 1062 cm^{-1} is corresponded to(–C-0-)stretching vibration.

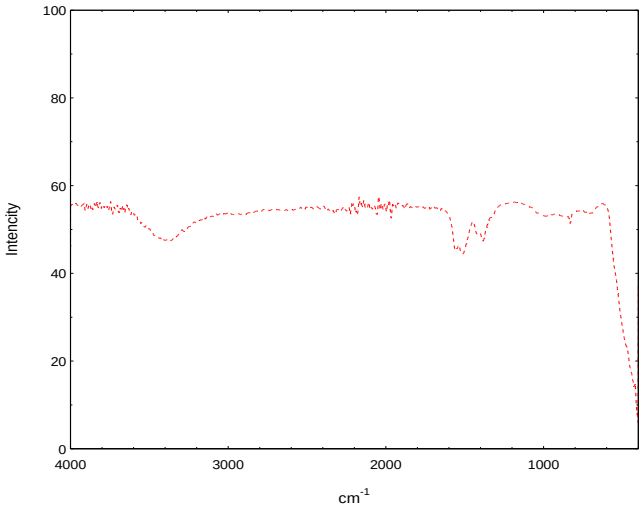

Fig6. Ftir of pure Zno shows the chracterstic vibrations.

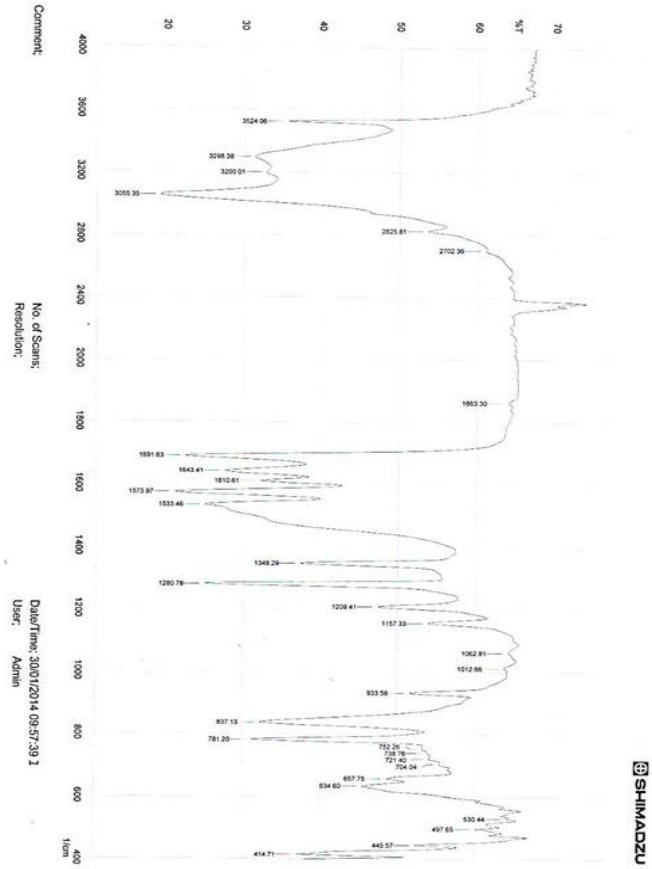

Fig7. Ftir spectrum of 5FC-ZnO-LH shows the chractertic vibrations of nanohybrids.

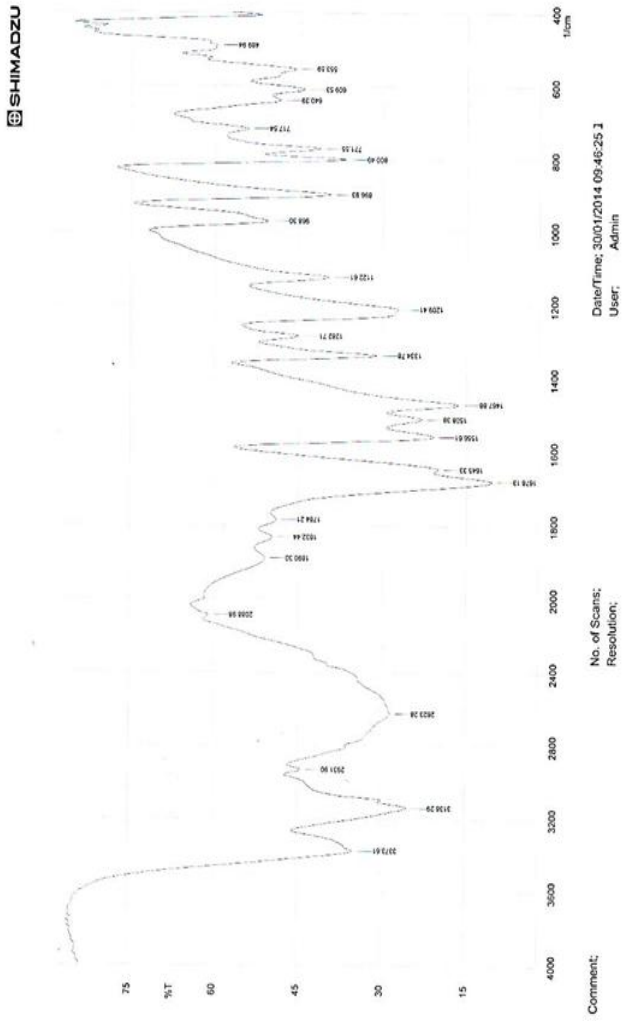

Fig5. Ftir spectrum of 5FC shows the chractertic vibrations Atomic forcing micryscope(AFM) study of the surface morphology:

C. Surface morphology studies: Atomic Force Microscopy image was recorded with scanning probe Microscopy (SPM-AA3000) instrument employing soft wear WSXM (nanotech). The glass slides were cut to1X2cm and clean by putting them in (1:1) ethanol: deionized water solusion and treated by ultrasonic (Ultrasonic FALC) instrument.

Fig(15) Shows AFM images of the 5FC-ZnO LHs nano particles synthesized with 5FC-ZnO-LHs, with a mean size of (120.8nm). The AFM observation is in good agreement with the data obtained by PXRD technique. (Fig.8 A , 8 B) in two dimensional) and in the three dimentional shows a fiber structures in the case of (5FC-Zinc) average diameter of 120.8nm . This was a good indication to the intercalation done.

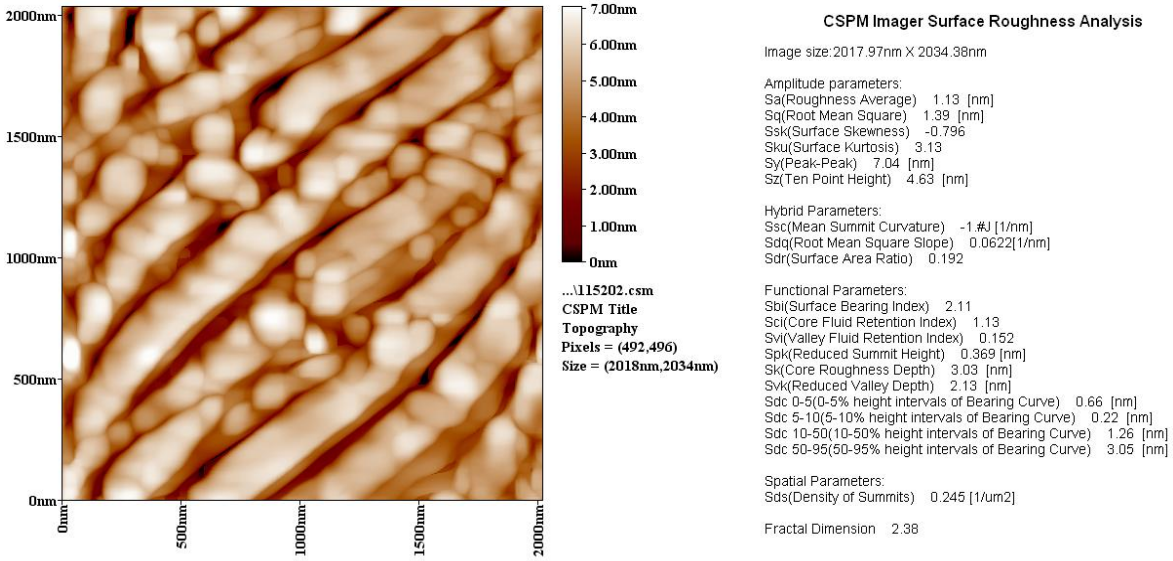

Fig8(A). Image of AFM shows the two dimentional coordinations of the 5FC-ZnO-LHs.

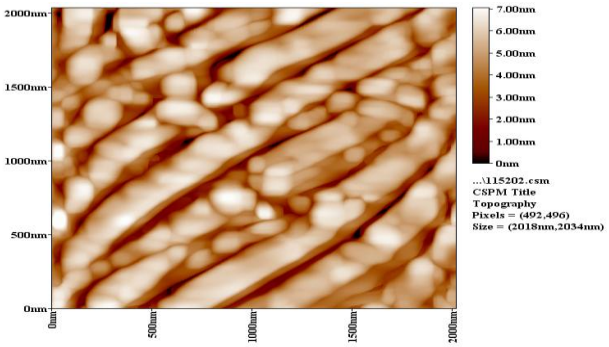

Fig8(B). Image of AFM shows the two dimentional coordinations of the 5FC-ZnO-LHs.

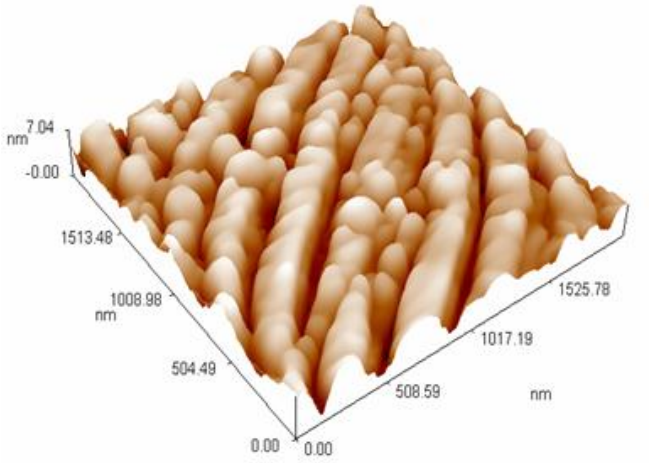

Fig9. Image of AFM shows the three dimentional coordinations of the 5FC-ZnO-LHs.

Avg. Diameter:120.82 nm

Table1. Shows the the pores distributions of granuals in the 5FC-ZnO-LHs nanohybrids

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
40.00	1.94	1.94	100.00	4.85	29.13	150.00	10.68	76.70
60.00	1.94	3.88	110.00	8.74	37.86	160.00	7.77	84.47
70.00	3.88	7.77	120.00	10.68	48.54	170.00	6.80	91.26
80.00	10.68	18.45	130.00	6.80	55.34	180.00	6.80	98.06
90.00	5.83	24.27	140.00	10.68	66.02	190.00	1.94	100.00

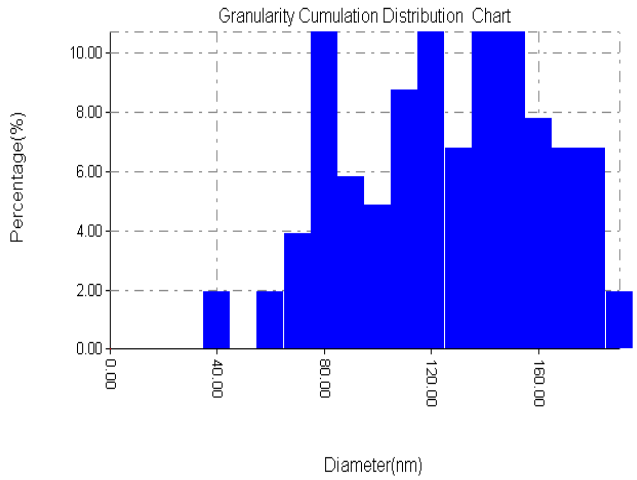

Fig10. Shows the histogram of the distribution of pores diameters.

D. Controlled Release of 5F-Cytosine (5FC) into Aqueous Media

The drug release properties of 5FC from the nanohybrid interlamellae. Into various aqueous media using (0.5 M of Na_2CO_3 have been conducted. "Figure (11) show the release profiles of composite in different aqueous solutions. The effects of various aqueous systems on the release of 5FC were evaluated according to the maximum accumulated release.

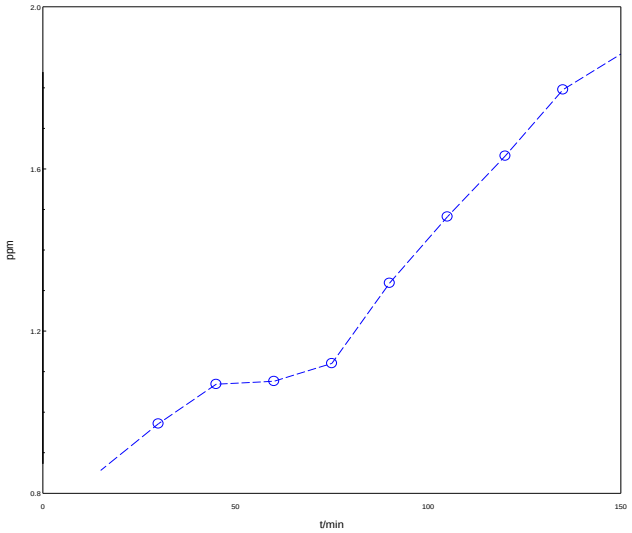

Fig11. Release in to carbonate media of 0.5M.

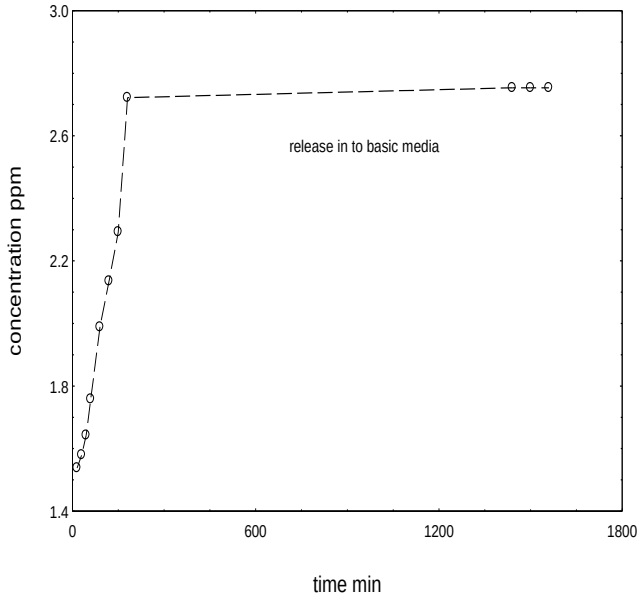

Fig12. Release into basic media.

It was observed that carbonate dominated the accumulated release percentage due to its affinity toward the interlayer of the layered hydroxides. As can be seen in "Figure (11), a rapid release of 5FC occurs at the initial stage, which is followed by a slower release of 5FC, 5FC is almost (97%)% replaced by CO_3^{2-}, resulting in the highest accumulated release among the media studied. The maximum release time shows that 5FC is exchange with CO_3^{2}at (180) min. All the previous discussions were about the 0.5M concentrations. The effect of concentration of aqueous systems on the release of 5FC was investigated according to the maximum accumulated release it was clear that the 0.5M aqueous system dominates the accumulated release percentage for all aqueous systems as shown in fig(11). It should be mentioned that the initial release rate of 5FC during the first (20) min in carbonate aqueous solution is much faster than that in the other aqueous systems. In the basic medium initial release is too slow but during the 180min the accumulation of 5FC is 60% (fig. 12), in the case of acidic the initial release is more than in the basic, the accumulation reached 50% during 1440min. fig(13)..

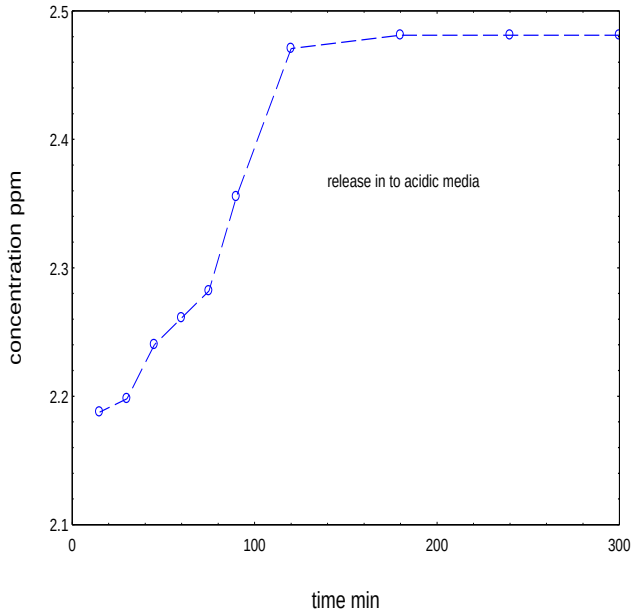

Fig13. Release of 5FC in to acidic media.

E. Release Kinetics

Release kinetics of 5FC has been evaluate~~d with~~ various models such as zeroth order, first order and pseudo-second order "Figure(14). The corresponding rate constants together with the r^2 values obtained from the fitting with zeroth, 1st, and 2nd order model. By comparing the correlation coefficient, r^2 values obtained from the fitting with those modules, it is clear that the release profile of 5FC from the nanohybrid was governed by the pseudo-second order kinetics modules in initial time till 80min then the release controlchange during the coming time as fig.(14) shows. The rate constant k obtained from the pseudo-second order kinetic model was more pronounced in case of 0.5 M carbonate solution, the nature of aqueous solution and the concentration of aqueous solution. Fig(15). Show the relation of 1st order reaction between

$$-\text{Log}\left(1-\frac{Ct}{Cf}\right)$$

versus time give or linear that Means the 1st order model does not good applying with the control release. Additionally, it can be seen from "Figure (14)" that the aqueous solution of 0.5M concentration can be considered more convenient to present the release process due to it could be released much more amount of 5FC intercalated .

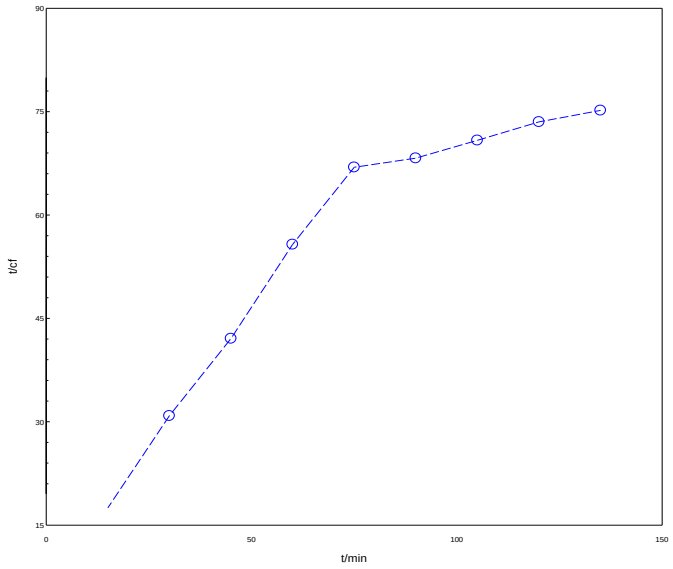

Fig14. Fitting 2nd order model to the release of 5FC in to carbonate media (0.5M).

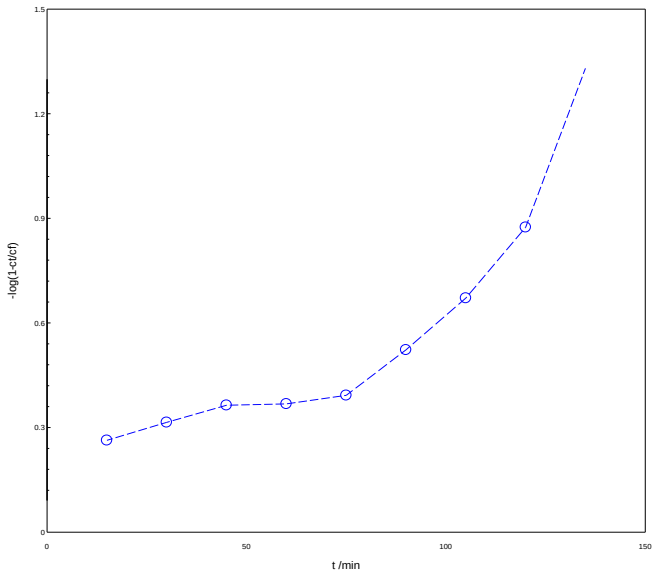

Fig15. Fitting first order model applying on the release of 5FC from the inter layer of 5FC-ZnO-LHs into carbonate media.

Results obtained from tables (2,3) showed that C. albicans was sensetive to the Nano hybrid (5-FC-Zno-LHs) more than 5-FC as free drug. Minimum Inhibitory concentration) MIC) were 5 mg /ml for all C.albicans isolate. (Fig.16) Results of statistical analysis showed of the table (2) there is significant differences ($p<0.01$) among isolates of samples wich studied from C. albicans, where, isolate N0. 2 was most sensetive toward the 5-FC-Zinc- LHs nanohybrid composite and gave highest of inhibition diameter for all concentration studied within nanocomposite , mean of inhibition diameter 12.033 mm, followed (4,3,5) without significant differences ($P>0.01$) among these three isolates, whereas, the isolation (NO.1) was lowest sensetive compare with the other isolates , the mean of inhibition diameter for isolates (NO.1) is 6.033 mm. Also, results of statistical analysis showed in table (2), that no significant differences ($P > 0.01$) for concentrations (1000, 500, 250 and 125) µg/ml compare with the control and among same this concentrations,where, this concentration not appear inhibition rate for all isolates. Whereas, increase in mean of inhibition diameter significante ($P<0.01$) for concentrations (5, 10, 15,20,25) mg. Of 5-FC-Zn-LHs nanohybrid. In general inceasing of nanocomposite concentration there is increase mean of inhibition diameter for all isolates.

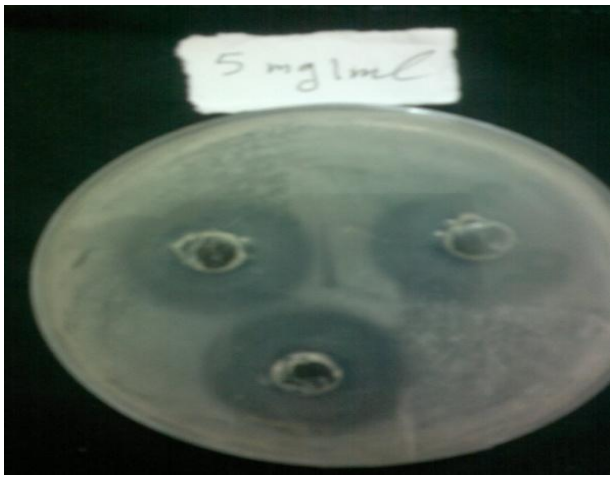

Fig23. Minimum Inhibitory concentration (MIC) of 5-FC-ZnoLH nanohybrid against Candida albicans yeast.

F. Biological activity

Table2. Inhibition activity for 5-FC-Zinc-LHs nanohybrid against C. albicans yeast.

Con. / isolate	Cont.	125 µg	250 µg	500 µg	1 mg	5 mg	10 mg	15 mg	20 mg	25 mg	Mean
1	0	0	0	0	0	8.33	10	12	14.33	15.67	6.033 A
2	0	0	0	0	0	19.33	24	24	26.33	26.67	12.033 C
3	0	0	0	0	0	16.67	19.33	21.33	24	26.33	10.766 B
4	0	0	0	0	0	10.67	21	23.33	25	28.33	10.833 B
5	0	0	0	0	0	13	15.33	17.33	27.33	29.33	10.232 B
Mean	0 a	0 a	0 a	0 a	0 a	13.6 b	17.93 c	19.59 d	23.39 e	25.66 f	

The Factor	Isolates	Concentration	Interference
$LSD_{0.01}$	0.369	0.522	1.167

* Nombers in table are indicate to mean of inhibition diameter for three duplicates
* Similar characters are indicating to no significant differences.
* Different character is indicating to significant differences.

While, results of statistical analysis showed of table (3) there is significant differences. (P<0.01) between isolates studed of C.albicans, isolate NO.4 was most sensitive for 5-FC m and gave highest inhibition diameter for all concentration studied of 5-FC, the mean inhibition diameter 2.932 mm, follow isolate NO. 3 with inhibition diameter 2.501 mm, then isolates NO. 2 and NO. 5, this isolates does not appear any significant differences among them (P>0.01), whereas, isolate NO.1 was lowest sensitive compare with the other isolates, the mean of inhibition diameter for isolate NO. 1 was 1.783 mm. Also, results of statistical analysis showed table (3), are no significant differences (P>0.01) for (1000, 500, 250, 125) µg/ml concentration which compared with control and between them, where does not appear any inhibition rate for all isolates. Whereas ,increas in mean of any inhibition significant (P<0.01) for (5,10,15,20,25) mg/ ml of 5-FC, there is increase in mean of inhibition diameter within increas concentration of composite .

Table3. Inhibition activity for free 5-FC drug against C. albicans yeast.

Conc. / isolate	Cont.	125 µg	250 µg	500 µg	1 mg	5 mg	10 mg	15 mg	20 mg	25 mg	Mean
1	0	0	0	0	0	1.5	3.33	3.67	4	5.33	1.783 A
2	0	0	0	0	0	0.67	2.33	15	5.33	6.33	1.966 AB
3	0	0	0	0	0	1.67	3	4.67	6.67	9	2.501 C
4	0	0	0	0	0	2.33	5.33	6	7.33	8.33	2.932 D
5	0	0	0	0	0	2.33	3.33	4	5.33	7.33	2.232 BC
Mean	0 a	0 a	0 a	0 a	0 a	1.7 b	3.46 c	4.67 d	5.73 e	7.26 f	

The Factor	Isolates	Concentration	Interference
$LSD_{0.01}$	0.369	0.522	1.167

* Nombers in table are indicate to mean of inhibition diameter for three duplicates
* Similar characters are indicating to no significant differences.
* Different character is indicating to significant differences.

IV. REFERENCES

[1] Albrecht, M.A., Evan, CW. And Raston, CL (2006). Green chemistry and the health implications of nanoparticles. Green Chem, 8: 417–43.

[2] Miyazaki, K. and Islam N.(2007). Nanotechnology systems of innovation – An analysis of industry and academia research activities. Technovation, 27: 661-671.

[3] Lv, L., Sun, P., Gu, Z., Du, H., Pang, X., Tao, X., Xu, R. and Xu, L (2009).Removal of chloride ion from aqueous solution by ZnAl-NO3layered double hydroxides as anion-exchanger. J. Hazmat. 161, 1444–1449.

[4] Nalawade, P., Aware, B., Kadam, V.J. and Hirlekar, R.S.(2009).Layered double hydroxides: A review. J. Sci. Ind. Res. 68, 267–272.

[5] Borm, P. J., Robbins, D. , Haubold, S., Kuhlbusch, T., Fissan, H., Donaldson, K., Schins, R., Stone, V., Kreyling, W., Lademann, J., Krutmann, J., Warheit, D., and Oberdorster, E. (2006).The potential risks of nanomaterials: a review carried out for ECETOC, Part. Fibre Toxicol. 3: PP. 11-46.

[6] Tjong, S.C. and Chen, H. (2004).Nanocrystalline materials and coatings, Mater. Sci. Eng., R, 45: 1–88.

[7] Williams D.(2008).The relationship between biomaterials and nanotechnology. Biomat erials doi:10.1016, J.biomaterials. 01: 003.

[8] Grunberg .E., Titsworth, E. and M. Bennett. (1964). Chemotherapeutic activity of 5-fluorocytosine.Antimicrob. Ag.Chemother. 1963.P.566-568.

[9] Duschinsky, R., Pleven, E. and Heidelberger, C. (1957). The synthesis of 5- fluoropyrimidines. Journal of the American Chemical Society, 79: 4559–60.

[10] Heidelberger, C., Griesbach, L., Montag, B. J., Mooren, D., Cruz, O., Schnitzer, R. (1958). Studies on fluorinated pyrimidines. II. Effects on transplanted tumors. Cancer Research, 18 :305–17.

[11] Grunberg, E., Titsworth, E. and Bennett, M. (1963). Chemotherapeutic activity of 5-fluorocytosine. Antimicrobial Agents and Chemotherapy 3: 566–8.

[12] Tassel,D. and Madoff, M. A.(1968). Treatment of Candida sepsis and Cryptococcus meningitis with 5-fluorocytosine. A new antifungal agent. Journal of the American Medical Association. 206: 830–2.

[13] Herbrecht,R.,Ninoix,Y.,Fohrer,C.(2005). Management of systemic fungal infections: Aiternatives to itraconazole. J Antimicrob chemother 56 :39-148.

[14] Fuerer,C.,and Iggo,R.(2004).5-fluorocytosine increase the toxicity of wnt-targeting replicating adenoviruses that express cytosine deaminase as late gene. Gene ther 11 :142-151.

[15] Polak, A. and Grenson, M. (1973). Evidence for a common transport system for cytosine, adenine and hypoxanthine in Saccharomyces cerevisiae and Candida albicans. European Journal of Biochemistry, 32: 276–282.

[16] Vazquez J.A., Sobel, J.D. (2003). Candidiasis. In: Clinical Mycology. Dismukes WE, Pappas PG, Sobel JD (Eds). Oxford University Press, Oxford, UK, 143–187.

[17] Bashi,a.M., Haddawi,S.M, and Mezaal, M.A. (2013 a).Layered Double Hydroxode nanohybrid Intercalation with Folic Acid used as Delivery System their Controlled Release Properties.Arab Jsci Eng. 38:1663-1680.

[18] Bashi ,A.M.,Hussein,M.Z.,Zainal,Z. and Tichit ,D.(2013 b).Synthesis and controlled release properties of 2,4-dichlorophenoxy acetate zinc layered Hydroxide nano hybrid Journal of solid state chemistry, 203:19-24.

[19] Hussein, M. Zobir, H., Norhya.ti, Y. Asmah, H.. And Zainal. Z.(2010).Synthesis and characterization of (4-(2,4-dichlorophenoxy butyrate)-Zinc.