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USING NANO-CHITOSAN BOUND TO SOME DRUGS AND STUDYING ITS PHYSICAL PROPERTIES AND BIOLOGICAL EFFECTIVENESS

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ABSTRACT

Chitosan, due to its molecular structure, can be dissolved well within a variety of solvents and a variety of biologics, such as acids like formic and lactic acid. , Another main use of chitosan-based nanoparticles involves the ability to withhold various drugs, organic compounds, and even inorganic analytes .In this work, a number of drugs were loaded onto chitosan nano polymer ciprofloxacin, Ibuprofen ,The physical properties of the prepared drug were studied, including its solubility in a number of different solvents (deionized water, ethanol, chloroform, acetone, dimethyl sulfoxide, hexane, Toluene). The absorbance of the drug release was measured using the UV-visible spectrum in two different Buffer solutions with . pH (2.2, 8) at a constant temperature of 310 K as a function of time (hour and day).The biological effectiveness of the prepared drug was studied in inhibiting some types of disease-causing bacteria.

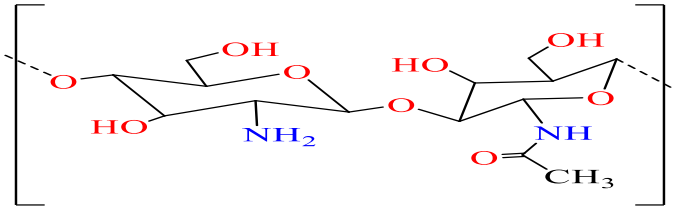
Keywords **:** *Polymer, Nano Chitosan, Antibiotic, solubility, absorbency, bacteria*

INTRODUCTION

There are various mechanisms for chitosan nanoparticle synthesis. These mechanisms include ionic gelation/polyelectrolyte complexation, emulsion droplet coalescence, emulsion solvent diffusion, reverse miscellisation .,Polymers: Biopolymers, synthetic polymers and their derivatives are commonly used in medicine and pharmacy as a result of significant advances in polymer chemistry and technology. Recently, special attention of scientists has focused on medical biopolymers, especially those used for drug delivery systems and therapeutic systems[1]. The polymer was used as a main tool in controlling the drug release rate and was also used as an agent to mask the taste[2]. It is also used as a stabilizing agent and a protecting agent in oral drug delivery[3]. Polymers are large molecules composed of small chemical molecules linked together by chemical bonds. The simple molecule that makes up a polymer molecule is called a monomer[4]. The process of linking this simple molecule together is called polymerization, which increases the molecular weight of the polymer.

Nano Polymer :The word nano is derived from the Greek word dwarf or small. Nano Polymer science is concerned with the study of polymer nanoparticles whose particle size ranges between (1-100) nanometers[5]. The transition from microparticles to nanoparticles leads to a change in their chemical and physical properties, and as a result they will possess properties and qualities that make them useful. In many advanced technology applications Nano polymers are used in medicine and pharmacy for the purposes of delivering medicine to infected cells without damaging healthy tissues[6]. They also help speed up the healing of wounds and skin burns, and also to detect infected cells and identify organ cancer[7].

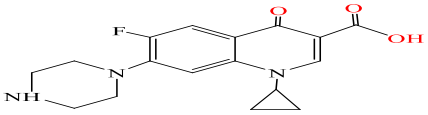
Nano Chitosan :It is a polymer composed mainly of chitin, which is extracted from the exoskeleton of arthropods, oysters and marine fish such as shrimp[8]. Its molecular formula is C56H103N9039 and its molecular weight is (1526.5 g/mol). It is a white to light yellow, odorless powder that is soluble in water and organic acids. Its melting point is 102.5 C[9]. It is a linear polysaccharide that contains B(1-4)-D-glucosamine. Acetylglucosamine[10], which is obtained by removing the acetyl group from chitin, According to the equation scales, this process is known as deacety[11] The following figure shows the structure of nano-chitosan:



Chitosan has many biological and chemical properties such as (easily absorbed, antibacterial, non-toxic, biodegradable) and therefore it is used for various medical purposes such as (antiacid activities, wound healing, anti-infective activities) and others. It is also used in industry and agriculture due to its high stability. Due to its non-toxicity and simple processing method, it has been used as a drug delivery carrier[12].

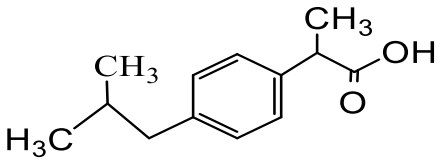
Drug delivery system : The drug delivery system includes the coupling between the drug and the polymer. This system is characterized by the fact that its release is controlled and differs from the traditional system, where the dose of the drug is released in the area to be treated[13], and the treatment remains for the longest period of time when it is taken and passes through the parts of the body without any effect until it reaches the place to be treated. For the patient, the frequency of dosing of the drug is also reduced, and as a result the toxicity of the drug is reduced, as are the changes in drug concentration in plasma levels, as their increase leads to the occurrence of other potentially harmful side effects. Or undesirable[14], but if the drug concentrations are lower, it does not provide sufficient therapeutic effectiveness and protects the drug substance from secretions. The intestinal tract effects and dose effect are highly specific, and this leads to high therapeutic efficiency [15]. The medications used in this study are:

Ciprofloxacin :It is an antibiotic that belongs to the fluoroquinolone family[16]. Its molecular chemical formula is (C17H18fN3O) and its scientific name is (cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid) a yellowish-white crystalline powder that is very slightly soluble in water, dried alcohol, and acetone. It dissolves well in acetic acid ,molecular weight is (331,346 g/mol), melting point is (320 C) , biological half-life is (4 hours)[17] and the chemical structural formula is as follows:



It is used to treat many bacterial infections, including urinary tract infections, respiratory infections, skin infections, cystitis, osteoarthritis, and many other types of bacterial infections[18].

Ibuprofen:It is a non-steroidal anti-inflammatory drug[19]. It is a monocarboxylic acid (propionic acid). Its molecular chemical formula is (C13H18O2) and its scientific name is -2-(4-(2-Methylpropyl)phenyl) propanoic acid , It is a white crystalline powder with a distinctive odor. It does not mix well with water, but it dissolves in alcohol, chloroform, ether, and acetone (, molecular weight are) 206.29g/mol) , meltin point is) 76 C (, biological half-life is (2-4 hours[20]) and its chemical structural formula is as follows :



It is used to relieve mild to moderate pain, especially headache, fever, menstrual cramps, arthritis, and toothache[21].

INSTRUMENTAL & MATERIALS:

1- Melting points were recorded using a device Electro thermal .VOL 15 Watt .45 W in our university

2- FT-IR spectra were recorded by using (FT-IR 8300 Shimadzu , Japan) in the range (400-4000) cm- 1 as KBr discs in our university .

3- 1H.NMR– Spectra , Bruker – Uitra Shield – 300 MHz Switzerland , with DMSO-d6) carried out in Tehran Universit .

4- UV – Vis spectrometer – (Shimadzu , Japan in our university)

5- An incubator for bacterial growth at the Exir Research Center

6- Cultivation media sterilization devic Hirayama (HVE- 50) Japan at the Exir Resarch Centr

Experimental Part:

Preparation of Nano Chitosan – Drug

1- Preparation of Compound (N1) :Ciprofloxacin drug (3.313 g, 0.01 moles) were dissolved in 30 ml THF with (3 drops) of concentration HCl and added to nano chitosan (1.5 g, 0.0000034 mole) and reflex for 24 hr. Finally the precipitate was washed by diethyl ether and 2.0 M NaOH and leave to dry for 16 hr.

2- Preparation of Compound (N4): Ibuprofen drug (2.062 g, 0.01 moles) were dissolved in 30 ml THF with (3 drops) of concentration HCl and added to nano chitosan (1.5 g, 0.0000034 mole) and reflex for 24 hr. Finally the precipitate was washed by diethyl ether and 2.0 M NaOH and leave to dry for 16 hr.

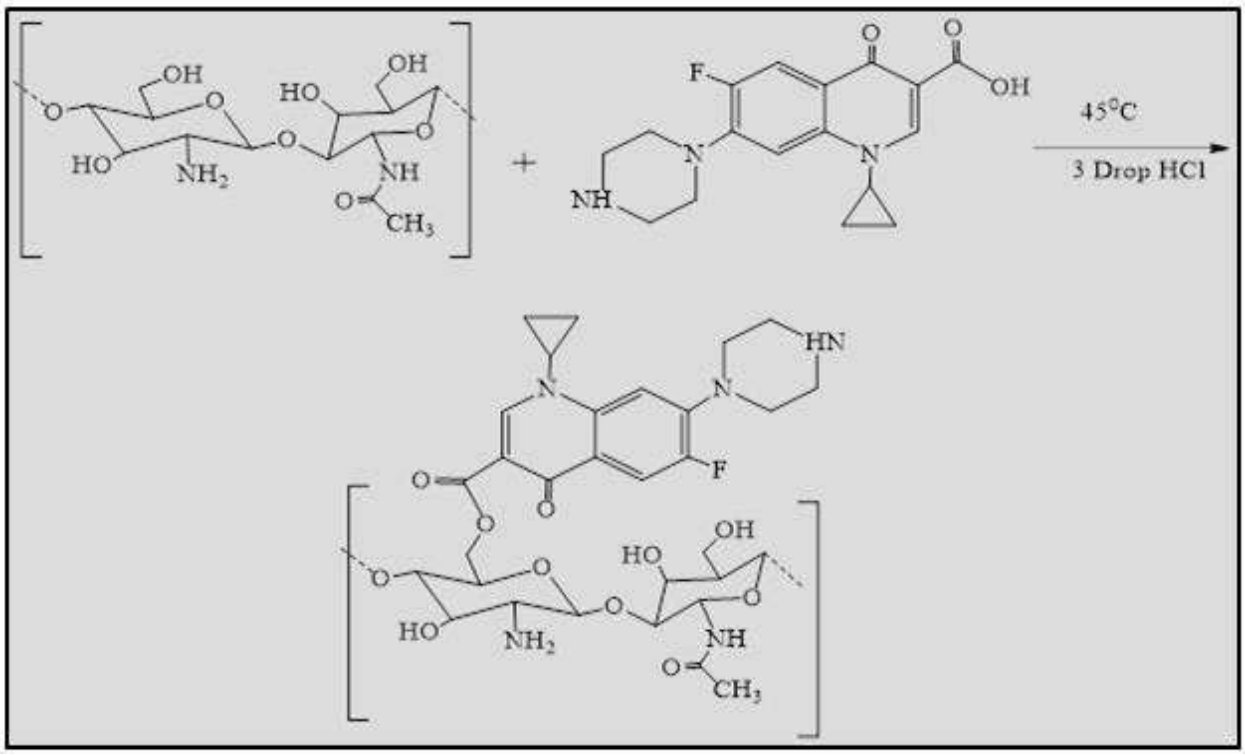


Photo. 1: The nano drug chitosan was prepared through the following reaction

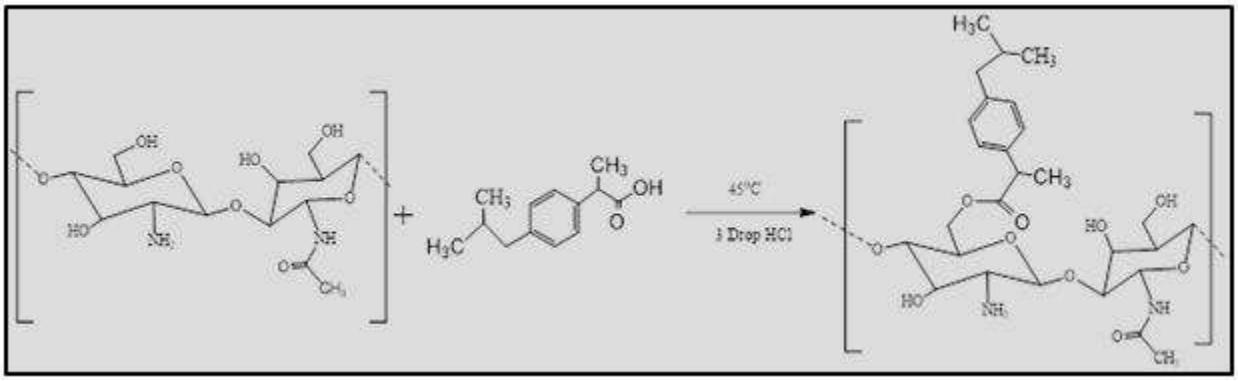


Photo. 2: The nano drug chitosan was prepared through the following reaction

Physical Characteristics of Nano Chitosan-Drugs Synthesis:
Studies have been conducted on the solubility and drug release characteristics of micro chitosan-drug compound, as demonstrated below:

1- **Solubility Characteristics:** The synthesized nano-chitosan (N1,N4) was placed in a test tube weighting 0.01 g. It was then attempted to dissolve in a variety of solvents, including ethanol, ether, chloroform, DMSO, hexane, and acetone. The solubility of the prepared drug chitosan was then assessed [22].

2- **Pharmacological Release from Nano Chitosan-Drug :**The amount of drug released from the manufactured nano chitosan-drugs was measured using a UV-Vis Spectrophotometer in tow distinct buffer solutions (2.2 , 8) at a constant temperature of 310 K (0.05 gm) for each type of nano chitosan-drugs that were placed in a 50 ml beaker. Measurements of absorption (controlled drug release) were made for a few hours at a time and for a few days[23].

Preparation of Buffer Solutions

Buffer solutions were prepared in the following manner [23,24]
1. pH=2.2: This solution was prepared, by mixing 500 ml of 0.2 M of KCl and 8.6 ml of 2.0 M of HCl.

2. pH =8: This solution was prepared, by mixing 500 ml of 0.025 M of

Borax [Na2B4O7.10H2O] and 0.5 ml of 0.1 M of HCl.

BIOLOGICAL EFFECTIVENESS:
The culture medium was prepared using special conditions, and the solutions whose biological effectiveness was to be evaluated were prepared at two different concentrations (1024ppm, 512ppm) against two types of bacteria [25], which are Gram-positive (Staphylococcus) and Gram-negative (Escherichia coli).

RESULTS AND DISCUSSION:
Organic Investigation N1: The FT.IR- Investigation : absorption band appeared at. (3446 cm-1) resulting from the vibration of the secondary amine group, and an absorption band at (3344 cm-1) that belongs to the hydrogen bond present in the alcohol (O-H) group, as well as The spectrum showed an absorption band at (3059 cm-1) representing aromatic (C-H), while the band (2877 cm-1) represents aliphatic (C-H). The spectrum showed an absorption band at (1764 cm-1) belonging to the carbonyl ester group. As for the absorption band (1647 cm-1). 1) It belongs to the amide carbonyl group, while the (C-O) band appeared at (1259 cm-1).

Organic Investigation N4: The FT.IR- Investigation: absorption band appeared at. (3441 cm-1) resulting from the vibration of the secondary amine group, and an absorption band at (3319 cm-1) that belongs to the hydrogen bond present in the alcohol (O-H) group, as well as The spectrum showed an absorption band at (2922 cm-1) representing aromatic (C-H), while the band (2881 cm-1) represents aliphatic (C-H). The spectrum showed an

absorption band at (1726 cm-1) belonging to the carbonyl ester group. As for the absorption band (1662 cm-1). 1) It belongs to the amide carbonyl group, while the (C-O) band appeared at (1263 cm-1).

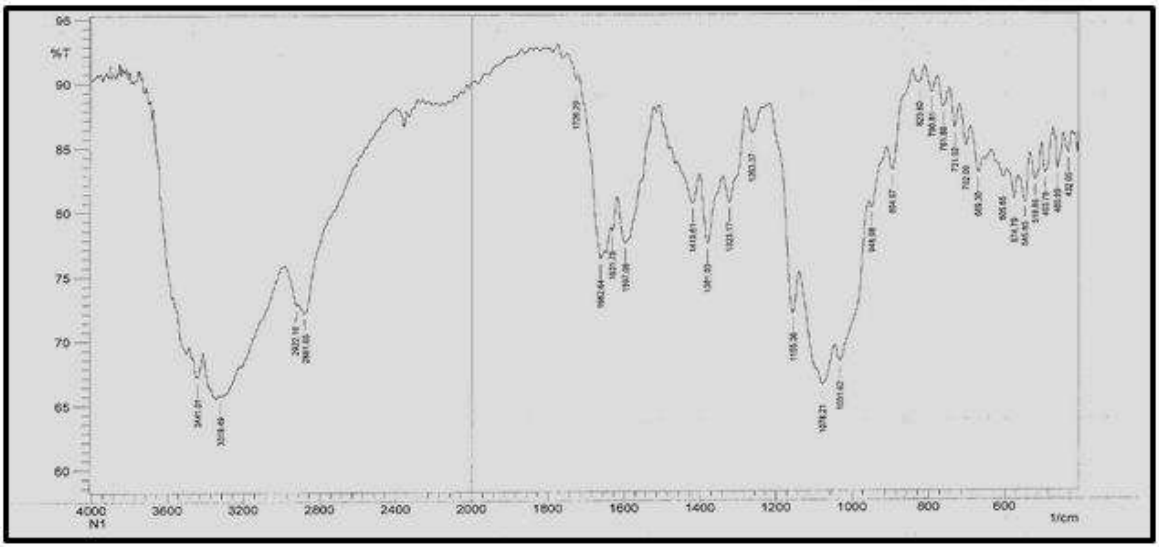


Fig (1): FT.IR of Compund (N1)

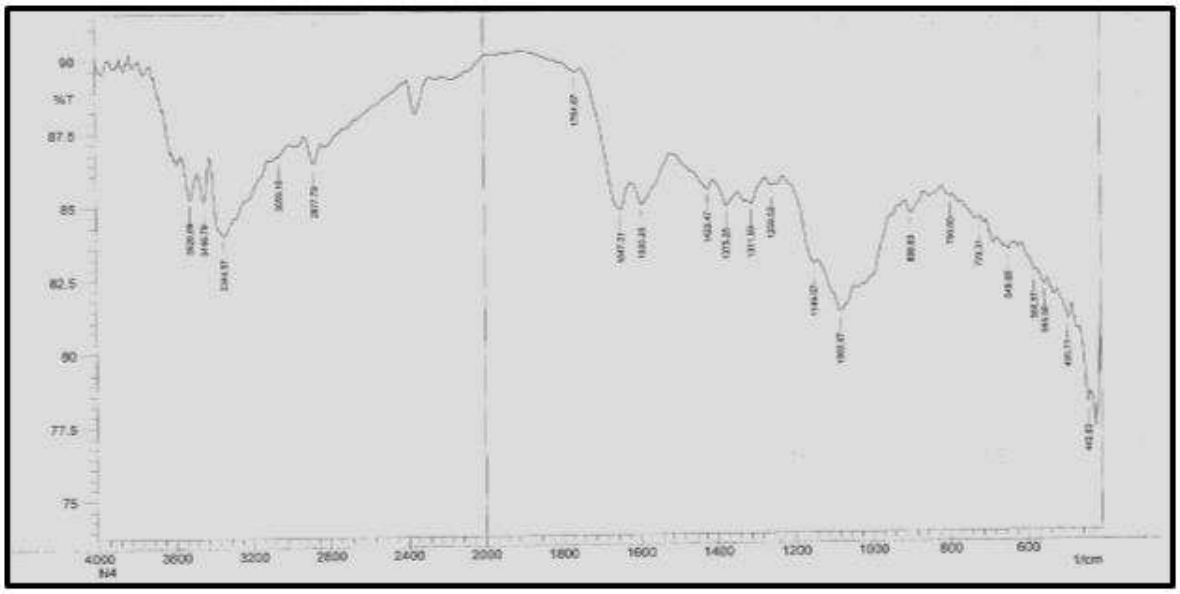


Fig (2): FT.IR of Compund (N4)

The 1H.NMR- Spectr of the compound N1 : where appearance signal of (OH group)at 8.7 ppm and appear signal of the H(NH amid) at 8 ppm and appear signal of the(aromatic – H) at 7.5 – 7.8 ppm and appear signal of(CH_2OH) at 5.3 ppm and appear signal of OCH2 at 5 ppm and appear signal of(OCH_3) at4.8 ppm and appear signal of(NH_2) at 4.5 ppm and appear signal of(DMSO)at at 2.5 ppm and appear signal of(CH_2) $_2$ at 1.5ppm and appear signal of (CH_3) at 1.3 ppm.

The 1H.NMR- Spectr of the compound N4 : where appearance signal of (OH group) at 8.7 ppm and appear signal of the(aromatic – H)at 7– 7.3ppm and appear signal of(CH_2OH) at 5.5 ppm and appear signal of OCH_2 at 4.8 ppm and appear signal of(OCH_3) at4.5 ppm and appear signal of (NH_2) at 4.2 ppm and appear signal of(CH_3)$_2$ at 1.8ppm and appear signal of(CH) at 1.5ppm and appear signal of (CH_3) at 1 -0.8 – ppm.

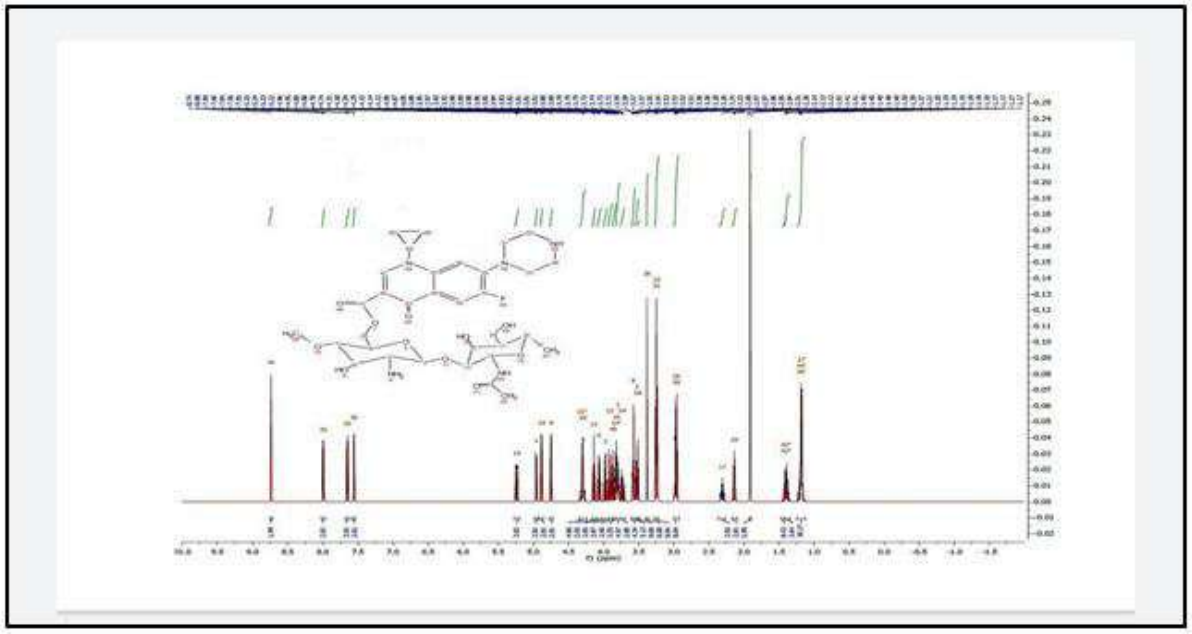


Fig (3) H.NMR of Compound [N1]

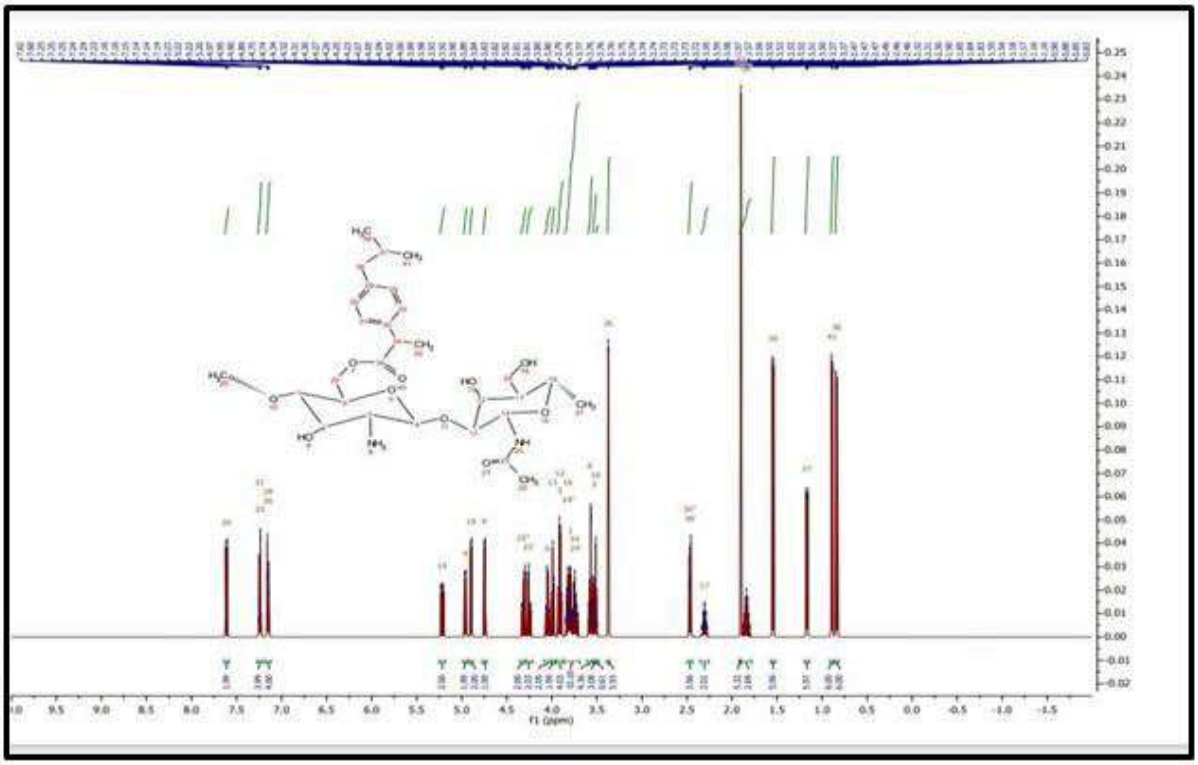


Fig (4) H.NMR of Compound [N4]

Physical Properties of Prepared Nano-Pharmaceutical Compounds:

1- Characteristic of Solubility :The solubility of these polymers was observed, where some of them were completely dissolved (+), some were molecularly soluble solids (partially), and the other was not completely dissolved (-).

Nano Chitosan drugs	H2O	DMSO	Acetone	Chloroform	ETOH	Toluene	Hexane
N1	Partial	+	-	Partial	+	-	-
N4		+	Partial	+	+	Partial	_-

The produced nano chitosan drug's solubility

2- **Drug Release** :Release the drug from the synthesized nano chitosan-drugs were measured in two distinct buffer solutions (2.2, and 8.0) at a constant temperature of 310 K using a UV-Vis Spectrophotometer. As shown in the following tables and figures:

A table showing the drug release time per (hour and day) from nano-chitosan drugs in PH =2.2 at 310 K

Time	Release of drug	
(Hour)	**N1**	**N4**
1	1.409	1.281
2	1.451	1.325
3	1.481	1.364
4	1.533	1.417
5	1.533	1.417
Day		
1	1.712	1.438
2	1.876	1.514
3	1.965	1.616
4	2.121	1.707
5	2.186	1.807
6	2.236	1.898
7	2.345	1.998
8	2.475	2.156
9	2.574	2.251
10	2.654	2.320
11	2.776	2.434
12	2.776	2.434

A table showing the drug release time per (hour and day) from nano-chitosan drugs in PH = 8 at 310 K

Time	Release of drug	
Hour	**N1**	**N4**
1	1.685	1.524
2	1.725	1.562
3	1.767	1.602
4	1.815	1.652
5	1.815	1.652
Day		
1	1.972	1.815
2	2.100	1.892
3	2.263	1.990
4	2.319	2.148
5	2.531	2.227

6	2.751	2.312
7	2.975	2.429
8	3.141	2.510
9	3.217	2.611
10	3.419	2.669
11	3.625	2.768
12	3.625	2.768

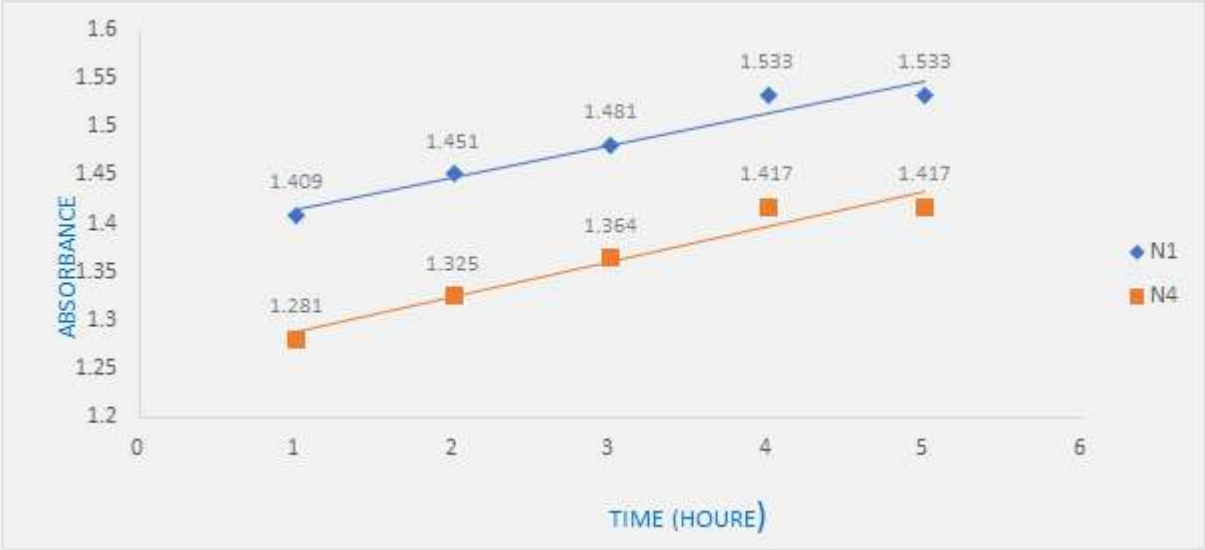


Figure: showing hourly drug release curves of chitosan nano drug at pH = 2.2 at 310 K

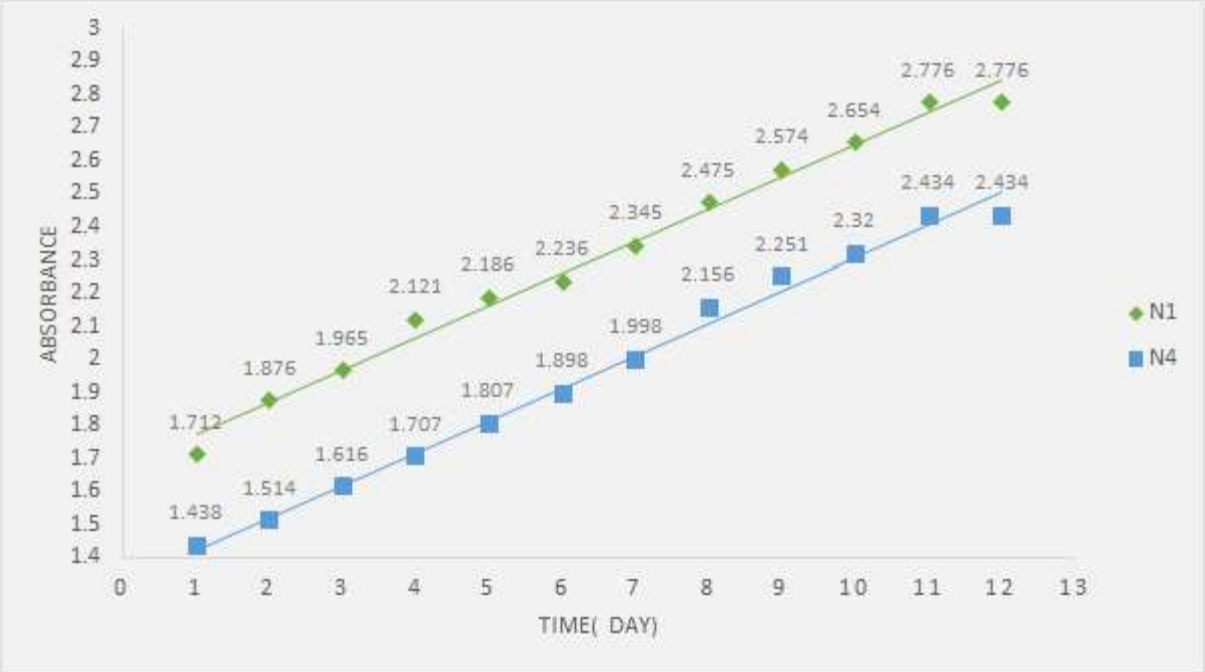


Figure: showing drug release curves per day for chitosan nano drug at pH = 2.2 at 310 K

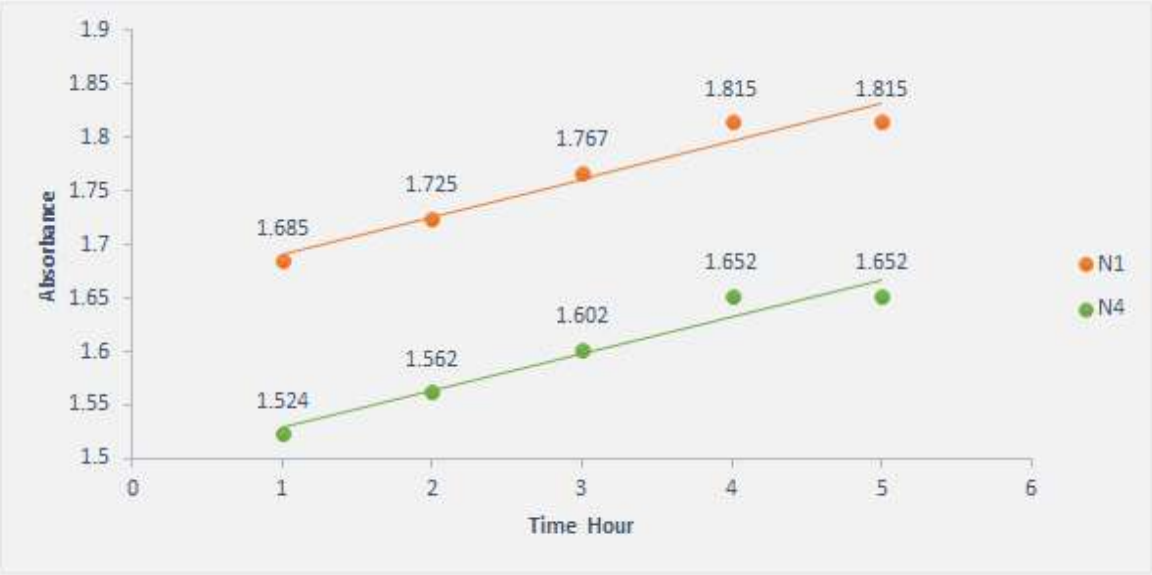


Figure: showing hourly drug release curves of chitosan nano drug at pH = 8 at 310 K

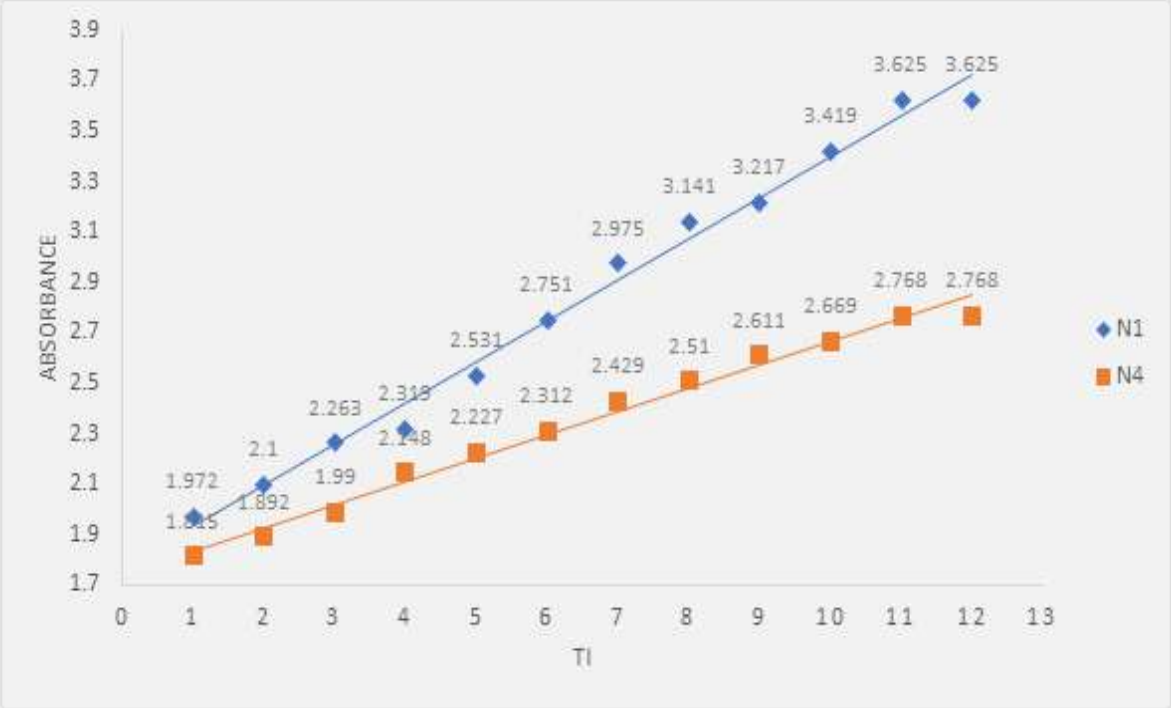


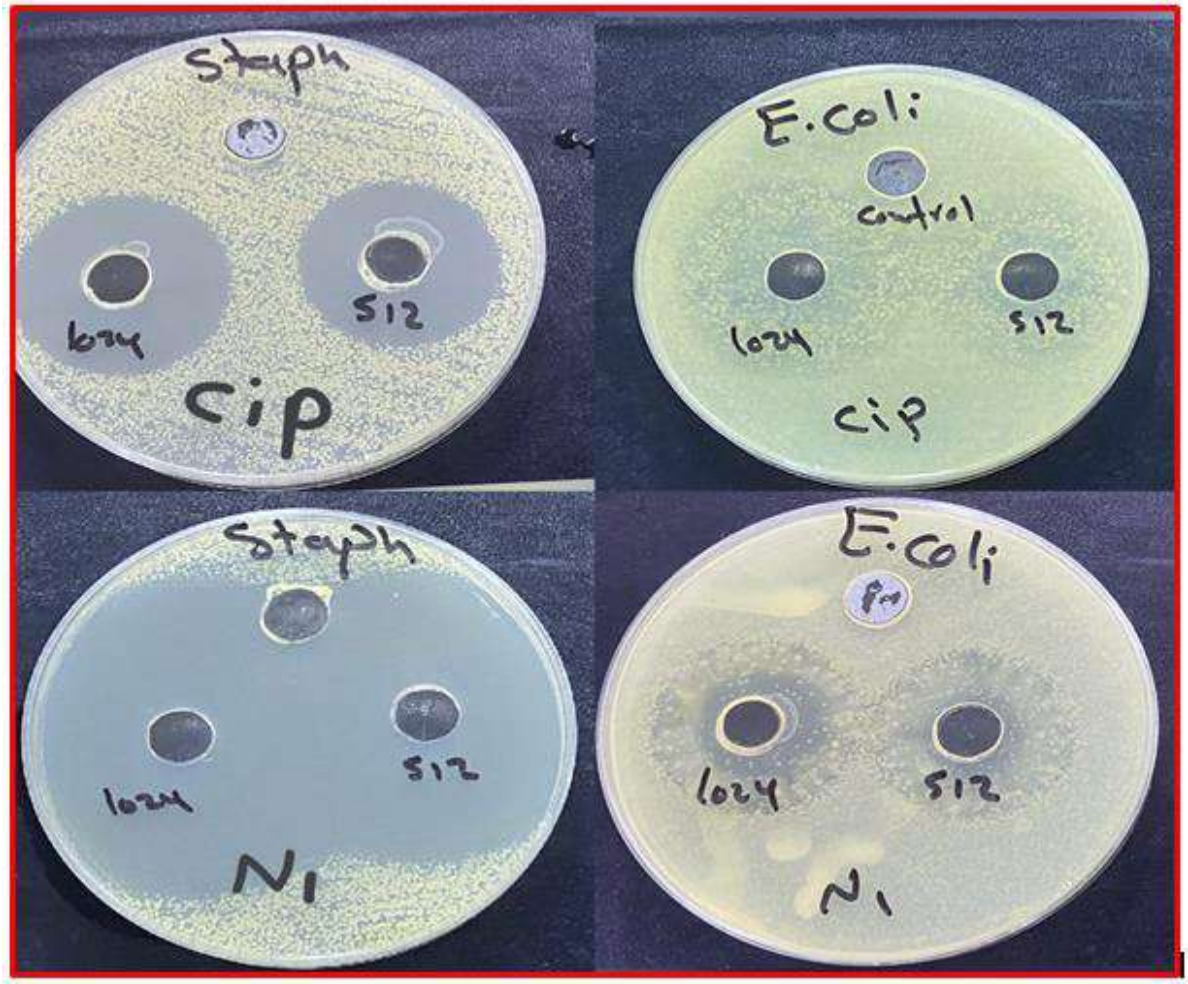
Figure: showing drug release curves per day for chitosan nano drug at pH = 8 at 310 K

DETAILS OF BIOLOGICAL EFFICACY RESULTS:

The results show that the effect of the prepared chitosan nanodrug gave a much higher result than antibiotics alone, as shown below.

A table showing the effect of the antibiotic (ciprofloxacin) compared to the prepared drug (N1)

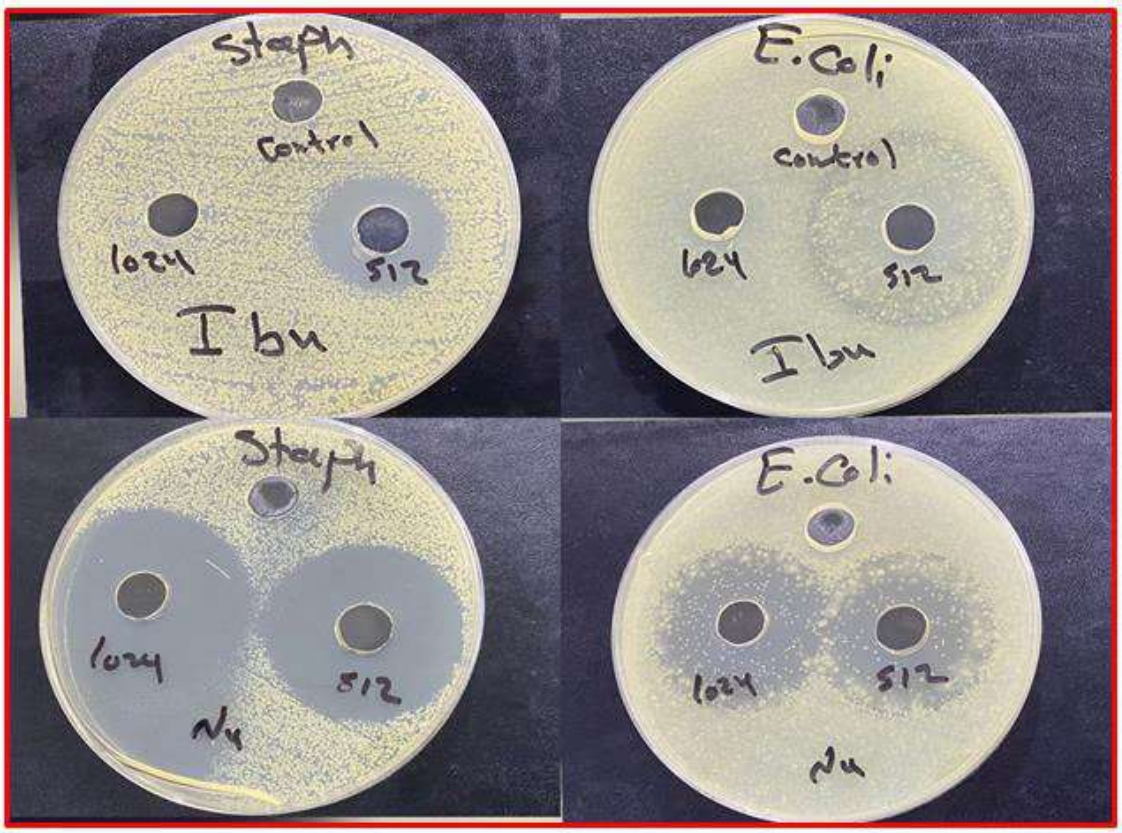
Type of bacteria	Ciprofloxacin		Drug of nano chitosan (N1)	
	1024ppm	512ppm	1024ppm	512ppm
Staphylococcus	20	18	40	40
Eschershia Coli	3	2	12	7



A figure: showing the effect of the antibiotic (ciprofloxacin) compared to the prepared drug (N1**)**

A table showing the effect of the antibiotic (Ibuprofen) compared to the prepared drug (N4)

Type of bacteria	Ibuprofen		Drug of nano chitosan (N4)	
	1024ppm	512 ppm	1024ppm	512ppm
Staphylococcus	Zero	8	25	23
Eschershia Coli	Zero	Zero	22	20



A figure: showing the effect of the antibiotic (Ibuprofen) compared to the prepared drug (N4

The results show that the effect of the prepared chitosan nano-drug gave a much higher result in inhibiting bacteria compared to using the drug alone when using two concentrations (1024 and 512) ppm, as was shown in the tables and dishes used above.

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