Synthesis of Ciprofloxacin Prodrug Chitosan

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Abstract

In this research a new prodrug polymer was prepared by reaction of ciprofloxacin as acyl chloride derivative with chitosan as amino. Polysaccharide gave new excellent biological properties and biomedical amide polymer Which could have potential use as a carrier for drug delivery system with ciprofloxacin formulation and evaluation of sustained release of ciprofloxacin prodrug chitosan was studied through amide attachment bond. It has been evaluated for all the necessary parameters like concentration variation and in vitro drug release had been conducted successfully, when in contact with acidic gastric content and release from the dosage as in the stomach-controlled condition. The prepared prodrug polymer was characterized by FTIR spectroscopy ,and UV spectroscopy ,physical properties were determined and intrinsic viscosity was measured .The good results were obtained as sustained release to prevent any side effect of ciprofloxacin with broad spectrum antibiotic . The biological assay was conducted for prepared prodrug using. The microorganism such as E.coli, staphylococcus aurous, the prepared prodrug appear high biological activity, compared with standard gentamycin.

Keywords: Prodrug, Chitosan, Ciprofloxacin.

الخلاصة

حضر في هذا البحث بوليمر دوائي جديد من تفاعل سبروفلوكساسين كمشتق الاسيل كلورايد مع الكيتوسان وهو بولي سكرايد اميني والذي اعطى مواصفات بالوجيه ممتازة وكدواء بايولوجي اميدي، والذي له امكانيه الاستخدام كحامل للدواء والتي بدورها تحرر السبروفلوكساسين بانتظام وبسرع متباطئة وقد حسبت كميه الدواء المتحرر من كيتوساتن سبروفلوكساسين واذي درست من خلال التحلل المائي للمجاميع الاميديه وقد درست قيم التراكيز المتحررة المختلفة مختبريا بنجاح , في المحيط المعيدي الحامضي و تحرر الجرع المحكمة في الظروف المعدية ايضا .شخص البوليمر الدوائي المحضر بواسطه مطياف الأشعة تحت الحمراء والأشعة الفوق البنفسجية ,وعينت الصفات الفيزيائية وقيست اللزوجة الجوهرية , وحصل على نتائج جيده لسرع التحرر الدوائي البطيء لتجنب التأثيرات الجانبية للسبروفلوكساسين كمضاد حيوي واسع الطيف .قيست الفعالية الباي لوجيه للدواء المحمل للأنواع البكتيرية مثل الكائنات الحية الدقيقة مثل القولونية، المكورات العنقودية الذهبية. وظهرت فعالية بالوجيه عالية بمقارنتها مع الجنتماسين القياسي.

الكلمات المفتاحية : دوائي، كيتوساتن، سبروفلوكساسين.

Introduction

Chitosan, chemically poly [(1-4)-β-2-amino-2-deoxy-D-glukosane] is N-deacetylated derivative of chitin. In comparison with chitin, it has better chemical and biochemical reactivity. It is composed from glucosamine units with free amino group on the second carbon. Its pKa is 6.3-7 [Schulz *et al.*, 1998], and salt form it has cationic character the amino group, which is rare in polysaccharides, can be used as the reactive

site. Natural cationic polymers are less abundant than anionic; therefore, chitosan attracts attention in various fields of use.

Chitosan, (poly-D-glucosamine), is a natural polymer derived from chitin, the second most abundant polysaccharide after cellulose. Chitosan is produced by alkaline deacetylation of chitin, by treating with 50% hydroxide for several hours or by enzyme hydrolysis of *N*-deacetylase. Degree of deacetylation of commercially prepared chitosan is usually in the range between 60-100%. In nature chitosan exists only in a small amount in several kinds of mushrooms i.e. aspergillus and mucor.

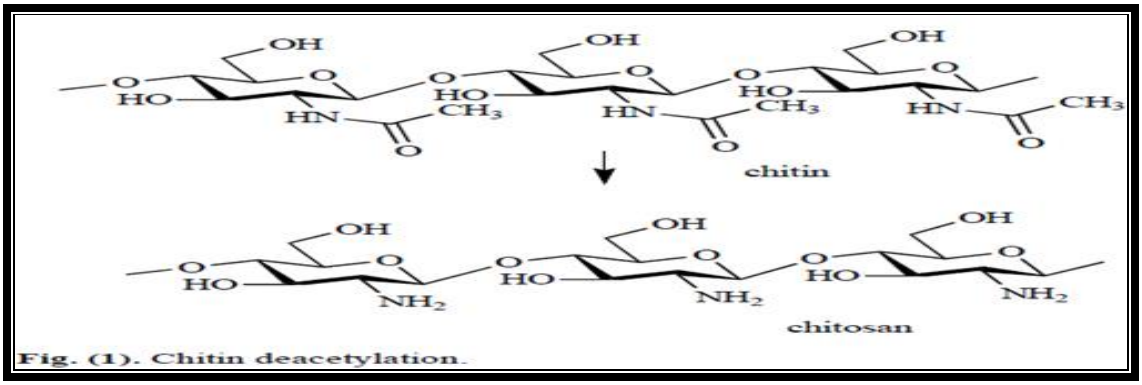


Fig. (1). Chitin deacetylation.

Chitosan has recently attracted more attention due to its significant biological functions such as biodegradability, biocompatibility, bioactivity and low toxicity [Thanou *et al.*, 2001; Yilmaz *et al.,* 2004]. These functions become better understood in addition to chitosan´s unique physicochemical properties. Chitosan is also bioactive agent useful in pharmaceutical and biomedical branches [Dodane *et al.*, 1998; Kato *et al*, 2003; Thanou *et al.*, 2005]. Chitosan could be useful especially as a supporter or carrier for biologically active species with control release of the drug in the target cell or tissue [Kumar *et al*, 2004; Felt *et al.*, 1998]. An optimal result should yield a minimum amount of side effects and prolonged activity.

Chitosan itself possesses antimicrobial activity against many G+, (*Staphylococcus aureus*, *S. epidermis*, *Bacillus subtilis*) Gbacteria, (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*) [Chun *et al.*, 1997] and fungi at pH < 6. Although the exact mechanism by which chitosan exerts its antimicrobial activity is not fully known, it has been suggested that the positive amino group of glucosamine units interacts with negative charged components in microbial cell membranes, altering their barrier properties, thereby preventing the entry of nutrients or causing the leakage of intracellular contents [Fernandez-Saizn *et al.*, 2006; Tsai *et al.*, 1999], which leads to cellule break up [Liu *et al.*, 2004; Helander *et al.*, 2001]. Another reported mechanism involves the penetration of low-molecular-weight chitosan in the cell, binding to deoxyribonucleic acid (DNA), and subsequent inhibition of ribonucleic acid (RNA) and protein synthesis [Zheng *et al.*, 2003]. Chitosan has also been shown to activate several defense processes in plant tissues and it inhibits the production of toxins and microbial growth because of its ability to chelate metal ions [Jia *et al.*,]. The biological activity of chitosan depends on many factors (its molecular weight, DE acetylation degree, chitosan derivatization, and degree of substitution, length and position of a substituent in

glucosamine units of chitosan, pH of chitosan solution) that lead to the extensive study of modifications in an effort to prepare suitable applications and form with improved activity on the target organism.

Ciprofloxacin hydrochloride, [Oliphant *et al.*, 2002] a fluoroquinolone, is the monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. It is a faintly yellowish to light yellow crystalline substance with a molecular weight of 385.8. Its empirical formula is C17H18FN3O3•HCl•H2Oand its chemical structure is as follows: in fig (2) this work aimed at synthesizing of ciprofloxacin carrier chitosan through amide bonds to obtain the new prodrug with controlled sustained drug release of a mutual drugs have the two biological action in the same time .

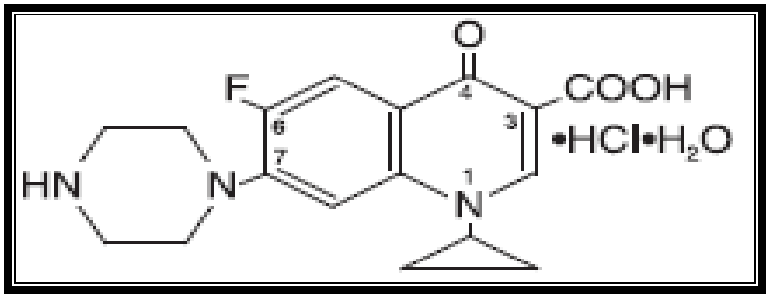


Fig (2) ciprofloxacin hydrochloride

Experimental work

Materials and Instruments

Chitosan was purchased from BDH, Ciprofloxacin was purchased from Aldrich, and DMF was obtained from Merck. All chemical materials were used without further purification.

FTIR spectra were recorded by a4300 Shimatzu Spectophotometer.UV-VIS.Spectra were recorded by Shimatzu.

Viscosity measurements

Ubbelhode, capillary viscometer was used to determine viscosities of prepared polymer at 25C.and the relative, specific, reduced and intrinsic viscosities were calculated from the intercept of graph by plotting **µ**red VS **C**%

Controlled release study(Felt *et al.*, 1998; Amita *et al.*, 2012)

A 100mg of modified polymer was kept in a cylinder containing of 100ml of buffer solution at 37 C˚ without stirring .The sample was periodically withdrawn and analyzed by UV.Spectrophotometer at suitable (271nm and 210 nm**)** for every prepared sample to determine the amount of the released Ciprofloxacin from prodrug, directly from the software built for many times using different pH values.

Synthesis of chitosan ciprofloxacin prodrug polymer [Bhatthatt *et al.*, 2013 Saboktakin*, et al.*, 2010]

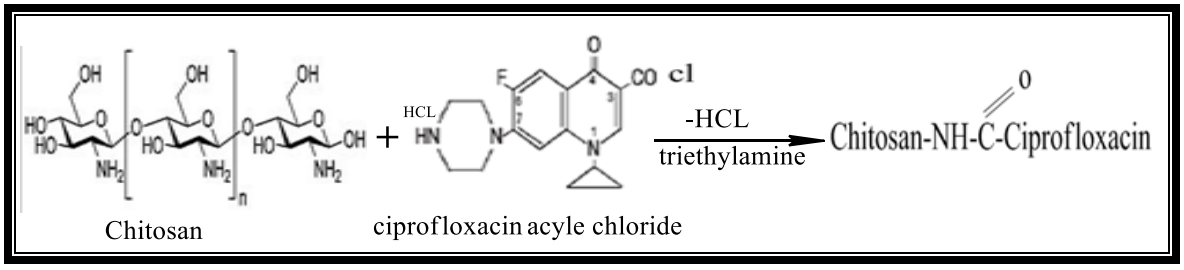
(1g., 0.018 mole)of ciprofloxacin was converted to its acyl chloride using 0.5ml of Thionyl chloride, the mixture was heated for about 20 mins.the excess of Thionyl chloride was distilled off. Then a (3g, 0.018mole) of chitosan which was solubilized in 5ml of 1%of acetic acid was added. with vigorously stirred about 2hr, the reaction mixture was heated at 40C˚ for 1hr.the modified polymer was formed, washed with5%of sodium bicarbonate solution, dried at 50C˚ under vacuum oven.

Table (1) physical properties of chitosan-ciprofloxacin prodrug polymer.

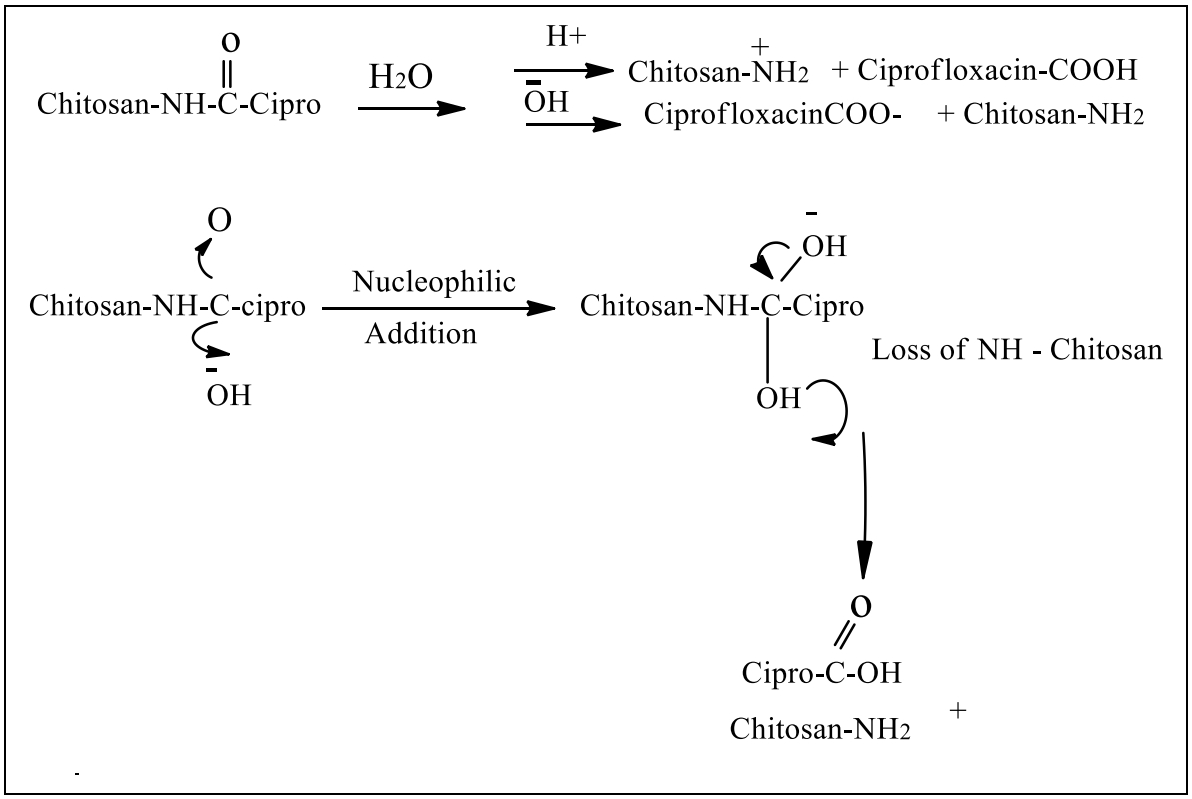
colour	Yield%	Softening point C˚	Intrinsic viscosity dl/g
yellow	82	>300	0.23

Result and Discussion

Chitosan is a natural polymer polysaccharide, which is available, sustainable, renewable, and possess. Better biocompatibility ,a low or non toxicity and have a higher modification capability compared to various synthetic materials ,these are due to containing NH_2 groups which could be substituted by ciprofloxacin acyl chloride, as shown in the following equation.



The aim of this work is to synthesize amide prodrug polymer which successful for long term drug delivery and highly desirable situation because it could be analyzed through amide group in different pH values at 37 C˚,it appears that the rate of analysis in pH 7.4is higher than the rate of hydrolysis in pH 1.1,this is attributed to $\bar{O}H$ as more nucleophilic attack than H_2O as illustrated in the following mechanism:-



Scheme (1) hydrolysis of amide prod

Fig (3a) uv.spectra of many samples of controlled drug release revealed the sustained drug release is higher than acidic media as shown in fig (3b) through alkaline medium pH 7.4.and fig (4) showed the relationship between mole fraction of released drug with time indicated the higher analysis than acidic medium this is attributed to higher nucleophilic attack on carbonyl group to enhance the hydrolysis of amide group to their corresponding amino and carboxylic compounds.

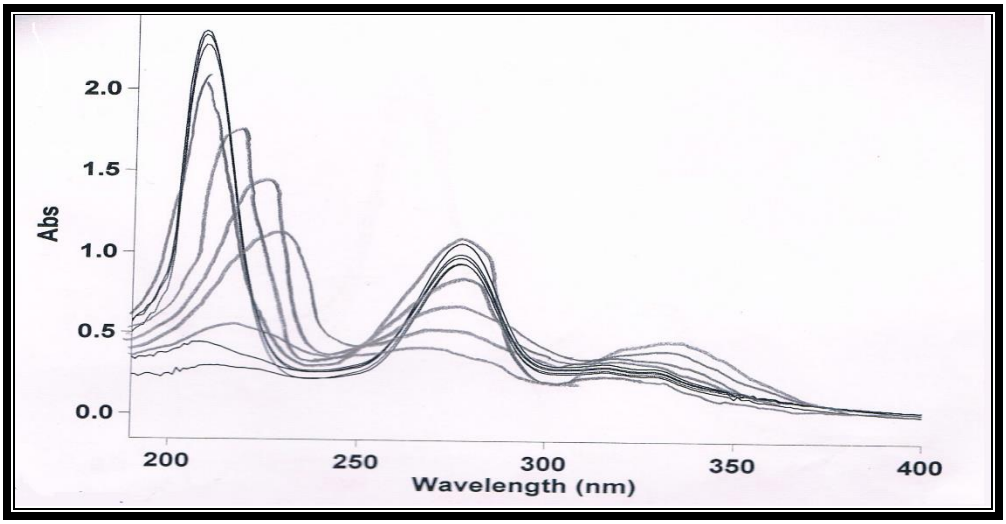


Fig (3a) UV.spectrum of chitosan-ciprofloxacin release in pH7.4 at 37˚C at λmax210 and 278nm

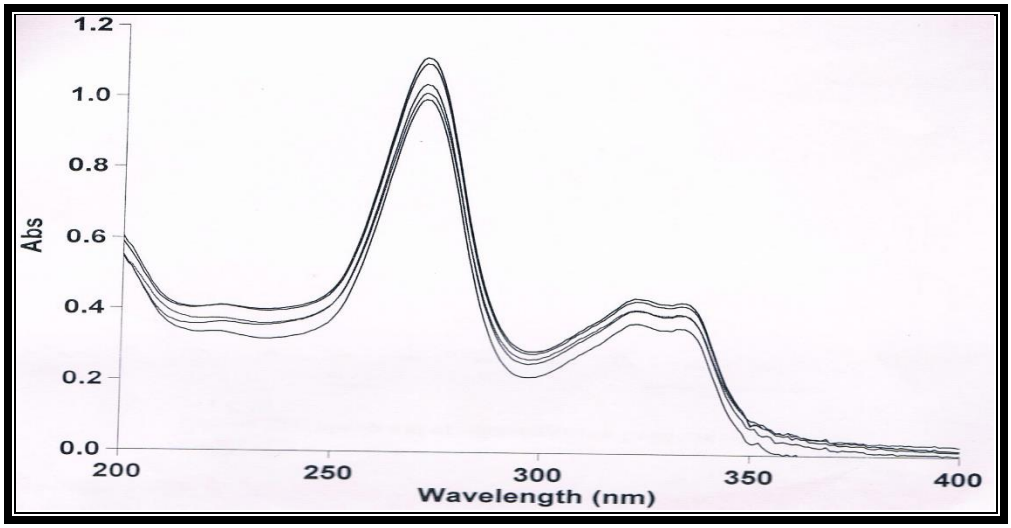


Fig (3b) UV.spectrum of chitosan-ciprofloxacin release in pH1.1 at 37˚C at271 nm

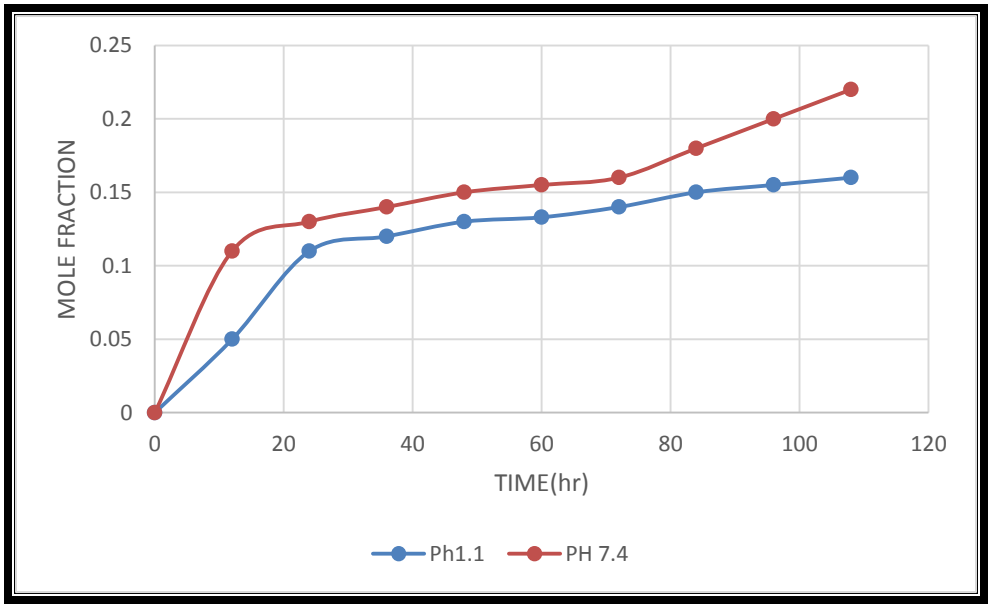
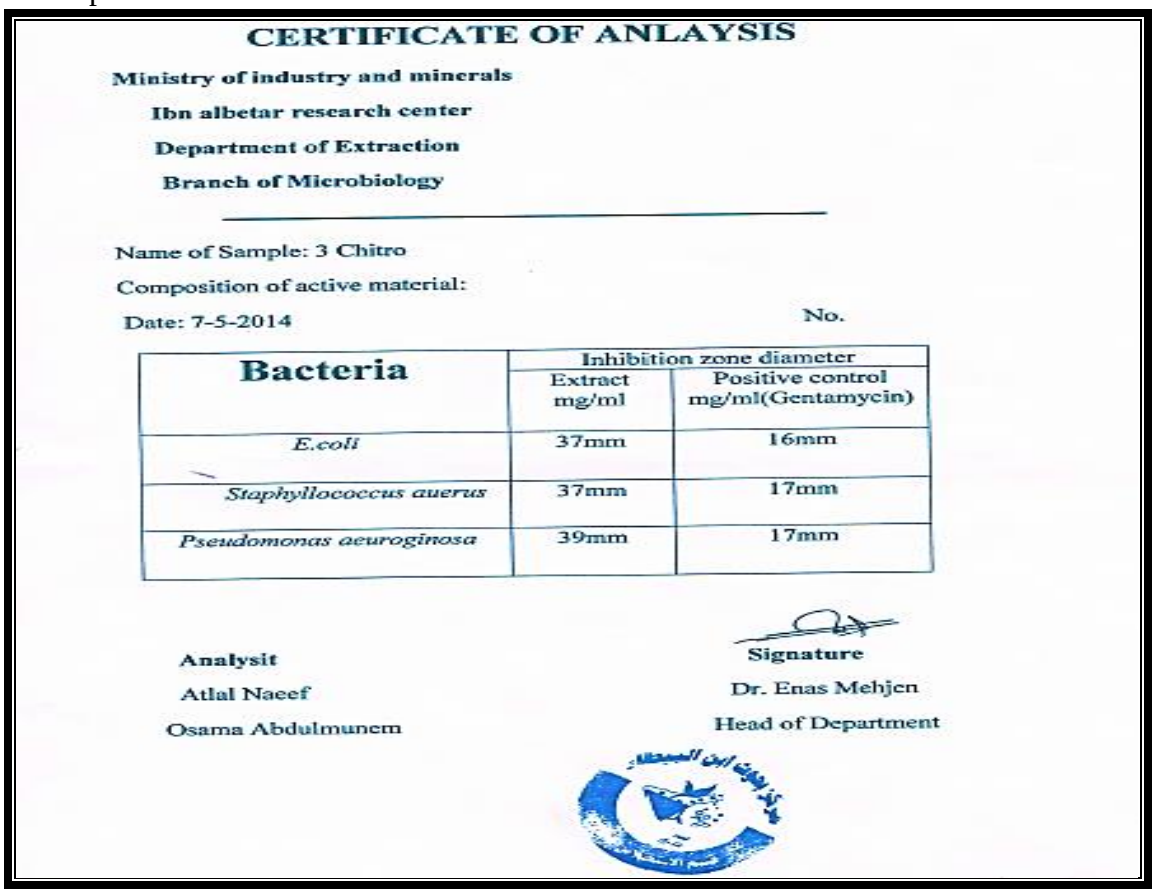


Fig (4) controlled ciprofloxacin release from amide prodrug chitosan in pH7.4 and 1.1 at 37˚C in 271nm

Antibacterial assay

The inhibitory effect was done for prepared Cipro chitosan prodrug in the growth medium at concentration mg/ml of nutrient agar in petri dishes. The agar was inoculated the bacteria plugged out front old culture of E.coli, staphylococcus and pseudomonas aecuroginosa,on nutrient agar the plate incubated at(37C˚) and the colony was estimated by measuring perpendicular diameter of colony, was compared with gentamycin, the analysis of variance to show the statistical significance of the data as shown in Table(2),it revealed high biological activity, it inhibits the growth of gram negative bacteria with

high effective of use as antibacterial medicine, also it is suggested that may play an important role in antimicrobial activity, with high successful control with more development as a new derivative.



CERTIFICATE OF ANLAYSIS

Ministry of industry and minerals
Ibn albetar research center
Department of Extraction
Branch of Microbiology

Name of Sample: 3 Chitro
Composition of active material:
Date: 7-5-2014 No.

Bacteria	Inhibition zone diameter	
	Extract mg/ml	Positive control mg/ml(Gentamycin)
E.coli	37mm	16mm
Staphyllococcus auerus	37mm	17mm
Pseudomonas aeuroginosa	39mm	17mm

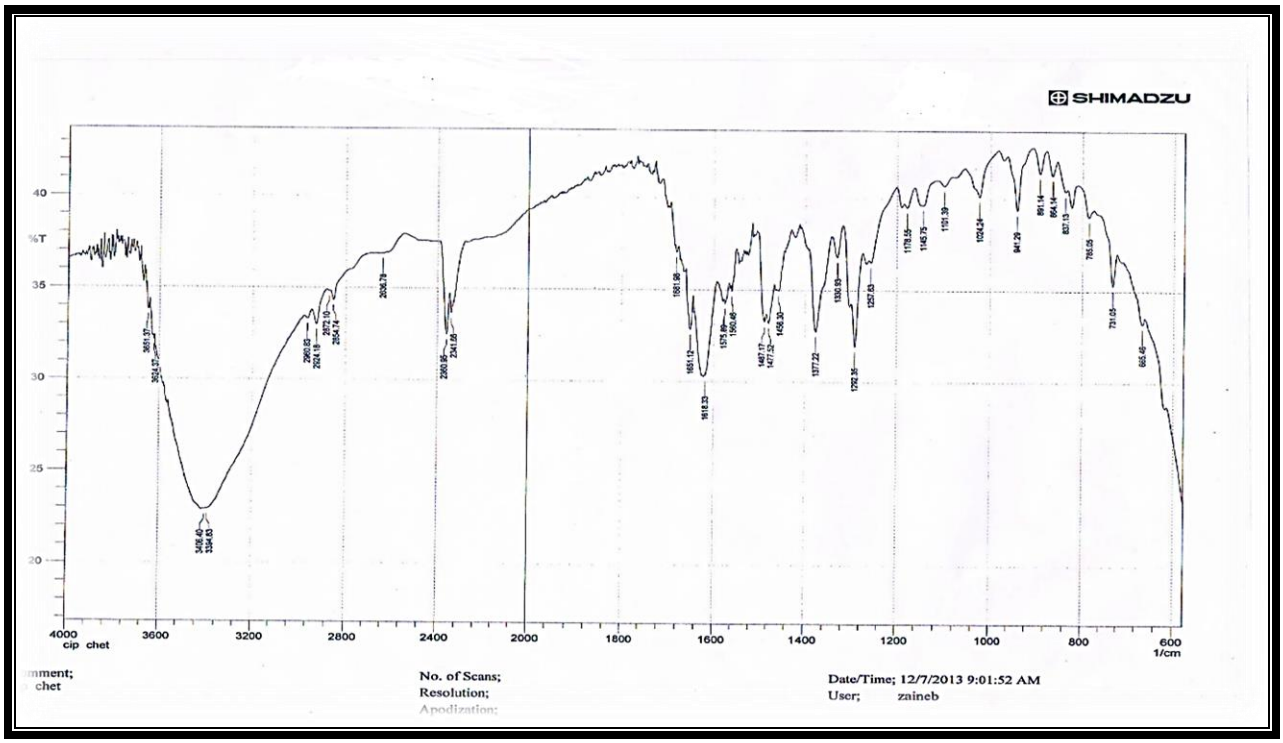
Analysit
Atlal Naeef
Osama Abdulmunem

Signature
Dr. Enas Mehjen
Head of Department

Biological assay for chitosan-ciprofloxacin prodrug

FTIR spectrum of prodrug polymer

Fig(4) showed the main absorption of formation amide group at 3200cm-1of NH stretching for an amide (shoulder)and 1651 cm-1 of C=O amide ,and 1618 cm-1 due to C=O of ciprofloxacin ,it was observed at 3400cm-1 the absorption indicated the remained some of $-NH_2$ stretching of unreacted in chitosan, the band at 2193 cm-1 which may correspond to the presence of C-N and3494 CM-1 due to OH and NH_2 of chitosan ,the C=C group was observed at 1600 cm-1 for aromatic ring in ciprofloxacin ,this indicated the formation of amide group and it was confirmed with the exhibited structure 1H-NMRspectrum was not analyzed clearly because of its difficulty and insolubility in DMSO



(Fig5) FTIR spectrum of Cipro chitosan prodrug polymer

Conclusion

It was concluded from this work that the chitosan carrier used has another biological active drug instead of inert molecule. The two pharmacologically active agent coupled so that each acts as promoiety for the other agent.

Chitosan and ciprofloxacin were selected because they have the same biological activity to give a mutual action agent with sustain controlled drug release to minimize the toxicity and to change the length of time of duration of action and to be specific site in the body in suitable pH values

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