Synthesis of N- Drug MaleamideChitosan

Drug polymers

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Abstract

In this research a new prodrug chitosan maleamide derivatives were synthesis via reaction of amino group of chitosan with maleic anhydride then converted to its corresponding acyl chloride, of the chitosan maleate derivative (A_1) was reacted with different drug-NH_2 (A_2-A_5) such as procaine, cephalexin, amoxicillin, and 4-aminoantipyrine.which could have potential use as a natural drug delivery system as a sustain release through hydrolysis of amide attachment in different pH values such as in acidic or basic medium. These derivatives were characterized by FTIR and ^{1}H-NMR spectroscopies. Controlled drug release was studied using UV. Spectroscopy at λmax 270nm gave a constant swelling percentage were measured indicated controlling release with other advantages and to minimize the toxicity and the side effect. Thermal analysis such as TGA and DSC were studied indicated the thermally stableprodrug polymers.

تحضير بوليمرات استر- اميد من بوليمر الكيتوسان الطبيعي

فريال محمد علي , سعدون عبد الله عودة , فارس حمود محمد

الكلمات المفتاحية:- بولي أميد-أميد، البوليمرات الدوائية الطبيعية المساعدة، التحرر

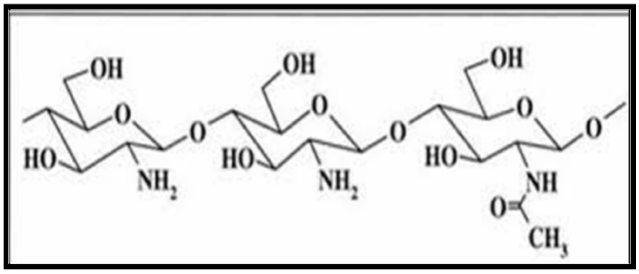
الخلاصة

حضر في هذا البحث مساعدات الادوية الجديدة من تفاعل الكيتوسان مع حامض الماليئك اللامائي من تفاعل المجموعة الامينية للكيتوسان مع حامض الماليتك اللامائي مكونا مشتق(A_1) الذي يحمل

مجموعة كاربوكسيلية حرة يمكن تحويلها الى كلوريد الحامض المقابل الذي تمت مفاعلتة مع بعض الادوية الامينية المختلفة مثل البروكائين، سيفالكسين، اموكسلين، 4-أمينو انتي بايرين (A_2-A_5) التي لها القابلية كأنظمة التحرر الدوائيالمحكم من خلال التحلل المائي لمجموعة الاميد، في دوال حامضية مختلفة حامضية وقاعدية بدرجة 37 م. شخصت المشتقات البوليمرية المحضرة بمطيافية الاشعة تحت الحمراء ومطيافية الرنين النووي المغناطيسي. ودرست سرع التحرر الدوائي المحكم باستخدام طيف الاشعة فوق البنفسجية عند طول موجي nm270 حيث اعطى تحرر دوائي بتراكيز ثابتة لفترة اربعة ايام، ودرست نسبة الانتفاخ المئوية والتي تؤكد التحرر البطيئ للدواء اضافة الى الفوائد الاخرى لتقليل السمية والمضار الجانبية، ودرست التحاليل الحرارية مثل التحلل الحراري الوزني والمسح المسعري التفاضلي الذي يدل على الثباتية الحرارية للبوليمرات الدوائية المساعدة.

Introduction

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine.The molecular weight of commercially produced chitosan is between 3800 and 20,000 Daltons. Chitosan exhibits excellent biological and economical properties for drug delivery systems[1] .It is non-toxic, biocompatible, and biodegradable and the source of its precursor, chitin is renewable, widely available material. Moreover, chitosan itself possesses bioactivity, such as antioxidant and antibacterial activities,chemical structure of chitosan is shown below



Recently the dissolution of chitosan in N-methyl morpholire N-oxide has been reported[3]. Controlled release technology emerged as a commercially sound methodology, and reproducibly release often agent into a specific environment over an extended period of time has many significant merits[4]. The most significant merit would be to create a desired environment with optimal efficacy[5,6] . The action of polymeric drugs invivo usually depends on hydrolytic on enzymatic cleavage of the drug moiety from the polymer [7].Polymers are becoming increasingly important in pharmaceutical applications especially in the field of drug delivery. Polymer was used as binder to increase viscosity and flow controlling agents in liquids,

suspensions and emulsions; can also be used as film coatings, to disguise the unpleasant taste of a drug, to enhance drug stability and, to modify the release characteristics [8,9].All controlled release systems aimed to improve the effectiveness of drug therapy[10]. This improvement can take the form of increasing therapeutic activity compared to the intensity of side effects, reducing the number of drug administrations required during treatment, or eliminating the need for specialized drug administration (e.g., repeated injections). Two types of control over drug release can be achieved, temporal and distribution control [11,12].

Experimental

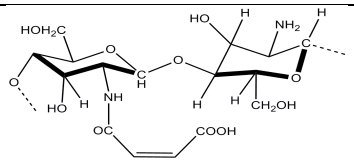
Instrumentation

Melting points were measured using Gallen Kamp M.F.B-600 melting point apparatus. (DSC) and (TGA) were recorded using (PL-STA 1500, RheometricSentific UK). The inherent viscosities were measured at 25 ℃. Swelling % of polymers wasdetermined by using 0.1g of polymer with water for 1 day. ^{1}H-NMR spectra were recorded on a Shimadzu spectrophotometer in Dimethylsulphoxide (DMSO). The FTIR spectra wererecorded by (4000-400cm^{-1}) on a Shimadzu spectrophotometer.Electronic spectra measurement using Cintra5-UV.Visble spectrophotometer. Chitosan, Maleic anhydride and Procaine were purchased from Fluka and 4-Aminoantipyrine, Amoxicillin, and Cephalexin were purchased from BDH. All available chemical reagents were used with a suitable purification.

Synthesis of (N-Maleamic acid –Chitosan) (A_1)

In a round bottom flask (0.87g,0.006 mole) of Chitosan was dissolved in 2 ml of 0.1N of acetic acid, the mixture was stirred about 2 hrs. Then(1.9 g, 0.006 mole) of dissolved maleic anhydride was added gradually with stirring. The mixture was isolated and washed with ethanol three times, then dried under a vacuum oven at 50℃. The physical properties were listed in table (1).

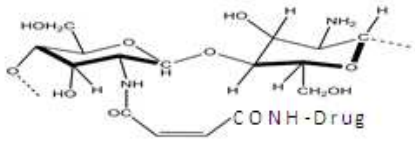
Table (1) Physical properties of prepared polymer (A_1)

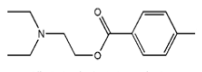
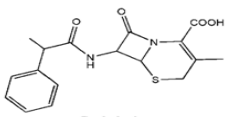
Comp No	-R	Color	M.P ℃	Viscosity η_{ln}= dl\g	Conversion%
A_1		gray	viscous	0.7	65

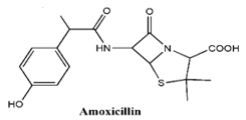
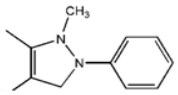
Substitution of Prepared (A_1) with Amino Drugs (A_2-A_5)[13,14]

In a round bottom flask equipped with condenser (2.24g, 0.01mole) of prepared A_2 was dissolved in 2ml of DMF add 10 ml of Dioxane, then 2ml excess of 0.01mol of thionyl chloride was added drop wise at 0℃ with stirring for 30 min. The orange polymer was isolated, then 0.01 mole of Drug-NH_2 such as Procaine, Cephalexin, Amoxicillin,or4-Aminoantipyrine), was dissolved in 5 ml of acetone, the mixture was heated at 60℃ for 1hr. The product was isolated as coogalate polymer, washed with ether and dried at 50℃ in vacuum oven.

Table (2) physical properties of drug polymers (A_2-A_5)



Comp No	- Drug	Color	softening° C	Viscosity η_{ln}= dl\g	Converted%
A_2	 2-(diethylamino)ethyl 4-aminobenzoate (Procaine)	Brown	275-290	0.76	60
A_3	 Cephalexin	Dark Brown	>300	0.75	62

A_4	 Amoxicillin	Yellow	280-295	0.72	63
A_5	 4-Aminoantipyrine	Yellow	270-290	0.73	64

Controlled Release Study [15]

A 50 mg of prepared polymer was kept in a cylinder containing 50ml of buffer solution and in a water bath at 37℃ without stirring. A sample from the release medium was periodically withdrawn and analyzed by UV. to determine the amount of the release. Mole fractions were constructed from UV. Spectrophotometer, atλ_{max} 270nm were determined directly through four days, in pH 7.4 and pH1.1 as shown in Fig.(13A and 13B) respectively.

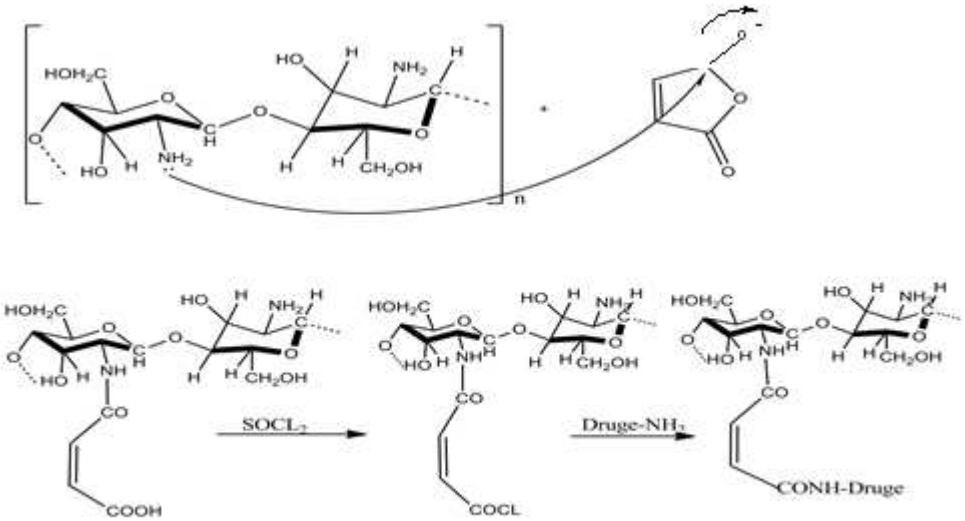
Measurement of Swelling % [16]

0.1gm of drug polymers were weighted accurately, and placed into flasks with 10ml solution of a given pH and kept in a thermostated bath at 37℃. Solutions with pH 1.2 (simulated gastric fluid), also pH 7.4 of (phosphate buffered saline) were measured at different times.

Swelling percentage of prepared polymers were studied in water according to S% = M_1-$M_\circ$ / $M_\circ$. 100. When $M_\circ$ is the weight of dry polymer at time $_\circ$. $M_1$is the swollen polymer in water at time t.

Results and Discussion

This review focuses on new drug polymers to development of drugs, to enhance their quality and to achieve the best drug delivery system; they are also desirable to release the drug molecules in specific site of the body for a longer duration, Chitosan was reacted with maleic anhydride produced compound A_1 then reacted with thionyl chloride converted to corresponding acyl chloride. Then substituted with drug amine such as procainethe mechanism is as shown below:-



A_1 Drug= Procaine, Cephalexin, Amoxicillin, 4-Aminoantipyrine A_2- A_5

Scheme (1) Synthesis of Drug polymers

FTIR spectrum, Fig (1) of polymer (A_1) showed the absorption bond at 1710cm^{-1} due to the C=O stretching vibration of the carboxylic groups. The absorption at 1637-1645 cm^{-1} was attributed to the formation amide groups. The other absorptions revealed at 3500cm^{-1} assigned to OH of maleic acid, and 2812-2921 cm^{-1} of C-H aliphatic.

FTIR spectrum, Fig (2) ofpolymer (A_2) showed the appearance of absorption at 3252 cm^{-1} assigned to —NH stretching of amide group, and as exhibit a at 1672cm^{-1} due to the C=O amide, and at 1714cm^{-1} due to C=O ester, 3010-3034cm^{-1} and 2858-2970cm^{-1} were a symmetrical and symmetrical stretching of C-H aromatic and aliphatic respectively indicated procaine amide prodrug polymer A_2.

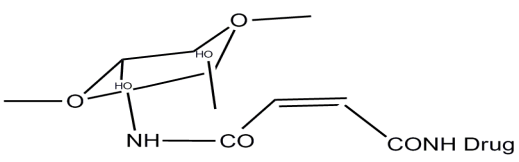
FTIR spectrum, Fig (3) of polymer (A_3) showed the absorptions of NH amide at 3190cm^{-1} and 1658 cm^{-1}of C=Oamide , 1778cm^{-1} of C=O ester, 1600cm^{-1} of C=C aromatic, at 3500cm^{-1} assigned to the OH carboxylic acid of drug.FTIR spectrum, Fig (4) of polymer (A_3) showed the appearance of absorption at 3400 cm^{-1} assigned to the OH phenolic of amoxicillin, and abroad bond at 3500-3000 cm^{-1} of OH stretching carboxylic group, and as exhibit a band at 3300 cm^{-1} due to NH amide and at 1681 cm^{-1} of C=O amide and 1749cm^{-1}of C=O ester. FTIR spectrum, Fig (5) of polymer A_4 showed the a symmetrical and symmetrical stretching of C-H aliphatic, 3012cm^{-1} of C-H aromatic, 1701cm^{-1} represented to stretching vibration of C=O amide, 2100 cm^{-1} which correspond to presence of C-N bond. Table (2) lists the main absorptions of prepared polymer (A_2-A_5). Fig (6) ^{1}H-NMR spectrum of polymer (A_1) showed the

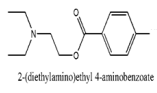
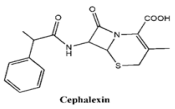
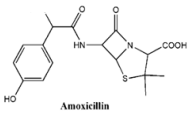
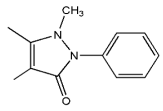
signal at 2.5 ppm assigned to (-CH-OH) 2H, doublet the signal at 3.2-3.8 ppm assigned to (CH-OH) doublet, at (4-4.3)ppm assigned to ($CH-NH_2$) Triplet, These signals assigned to chitosan signals. The maleamideattached with chitosan was appeared the signals at (6.1-6.9) ppm assigned to $(-CO-HC=CH-CO)_2$signals as singlet, at 13 ppm assigned to OH carboxylic acid of maleic acid. Fig (7) ^{1}H-NMR spectrum of polymer (A_2) showed the same signals were appeared in fig (6) of N-maleic chitosan signals which indicated the disappearance of signals at 13 ppm of OH carboxylic acid which converted to amide which appeared at 10.5 ppm thought procaine signals were showed the signal at 1.1 ppm assigned to $2CH_3$ (6H) Triplet, at 2.6ppm and 3.4 ppm assigned to ($O-CH_2-CH_2-N$) as (4H) Triplet, at (4.3-4.2) ppm assigned to 2($N-CH_2-CH_2$)quartet, at 6.4 ppm of 2H ortho aromatic procainamide, doublet, at (7-8) ppm assigned to m, m of procainamide as double doublet. The remained OH carboxylic appeared the signals at 10.5 ppm.

Fig (8) ^{1}HNMR spectrum of (A_3) showed the signalsindicated the chitosan and maleic attached with drug, the some signals were showed in fig (6), although the signals appeared at (4-4.6) ppm due to ($=C-CH_3$) as a doublet, at 3.5 ppm assigned to (CH_2) singlet, at 4.9 ppm assigned to (-CH-S) as a doublet

Fig (9) ^{1}H-NMR spectrum of polymer (A_3) indicated the signals of chitosan and maleamide as shown in fig (6), in addition to the amoxicillin signals which appeared at 1.1 ppm assigned to ($2CH_3$) singlet, at 3-2 ppm assigned to (N-CH-COOH) singlet, at 4.1 ppm assigned to (CH-CH-S) doublet, at (6-7.8) ppm assigned to (4H) aromatic o, o and m, m respectively, at 8.9 ppm assigned to NH amide and at 9 ppm assigned to OH phenolic . Fig (10) ^{1}H-NMR spectrum of polymer (A_4) was indicated the same signals of chitosan maleamide in addition to signals appeared at 2.1 ppm assigned to (5H) aromatic of 4-aminoantipyrine, at 3.9 ppm assigned to 3H of ($C=C-CH_3$) singlet.

Table (3) Spectral data for compounds (A_2-A_5)



Comp No	-Drug	υOH cm^{-1}	υNH cm^{-1}	υCH ar cm^{-1}	υCH al cm^{-1}	υC=O ester cm^{-1}	υC=O amid cm^{-1}	υC=C Ar cm^{-1}	υC-C Al cm^{-1}	υC-O cm^{-1}
A_2	 2-(diethylamino)ethyl 4-aminobenzoate (Procaine)	3450 OH chanson	3240	-3034 3010	2858-2970	1714 Ester	1658 Amide -amide	1599	1408	1255
A_3	 Cephalexin	3500 Broad-OH carboxylic acid	3190	3032	-2960 2854	1778	1695 Lactam 1658 amide	1600	1496	1116
A_4	 Amoxicillin	3500 OH carboxy 3450lic phenol	3300	3082	-2960 2864	1749	1734 lactam 1658 amide-amide	1600	1514	
A_5	 4-Aminoantipyrine	-	3250	3012	-2972 2893	-	1701 Cyclic amide 1658 C=O amide-amide	1614	1492	1255

Thermal analysis of prepared novel polymers were recorded, Fig (11A) showed TGA for (A_2) indicated its thermal stability through three stages, at 140.5℃ with weight loss 2%, at 279℃ with weight loss 75% and 370℃ with weight loss 95%.

Fig (11B), DSC thermogram of polymer (A_2) showed three endothermic degradation at 113.2 ℃, 167℃ and 235 ℃.

Fig (12A) UV spectra of controlled drug release, of polymer (4) the hydrolysis solution was detected by UV spectrophotometer atλ_{max}270nm pH 7.4at 37℃ indicated prodrug release gradually under mild conditions. The order of hydrolytic rate of the polymer was as in basic medium more than acidic pH 1.1 due to cleavage of drug-NH_2. The electropositive charge of the polymer chains cause both intermolecular and intermolecular repulsion and the H^+ ions attacks the carboxylic amide groups and the reversibility of acid catalyzed hydrolysis in acidic media. as shown in Fig(12B).Also the cleavages of amide bonds were compared in a phosphate buffer solution. In vitro studies showed the potential utility of the prodrug polymer a macromolecular have therapeutic efficiency of the physiochemical rate of the drug regeneration with a suitable specific site.

Conclusions

This strategy focused on new prodrugs for assuring the slow release to introduce a long-chain aliphatic amide to slow the hydrolysis it is useful for the treatment of psychoses through requirement of medication for extended periods. we could designed some prodrugs which could be efficient and selective on their site and metabolized to non-toxic derivative, also they a achieved the best drug delivery system, through the slow rate of swelling properties which ranged between12-20% as carried out in water. Chitosan was modified with spacer maleamide which attached with amino drug.Wich could increase the chemical properties of drugs as natural polymer, biodegradable, safe and non-toxic and other pharmaceutical properties

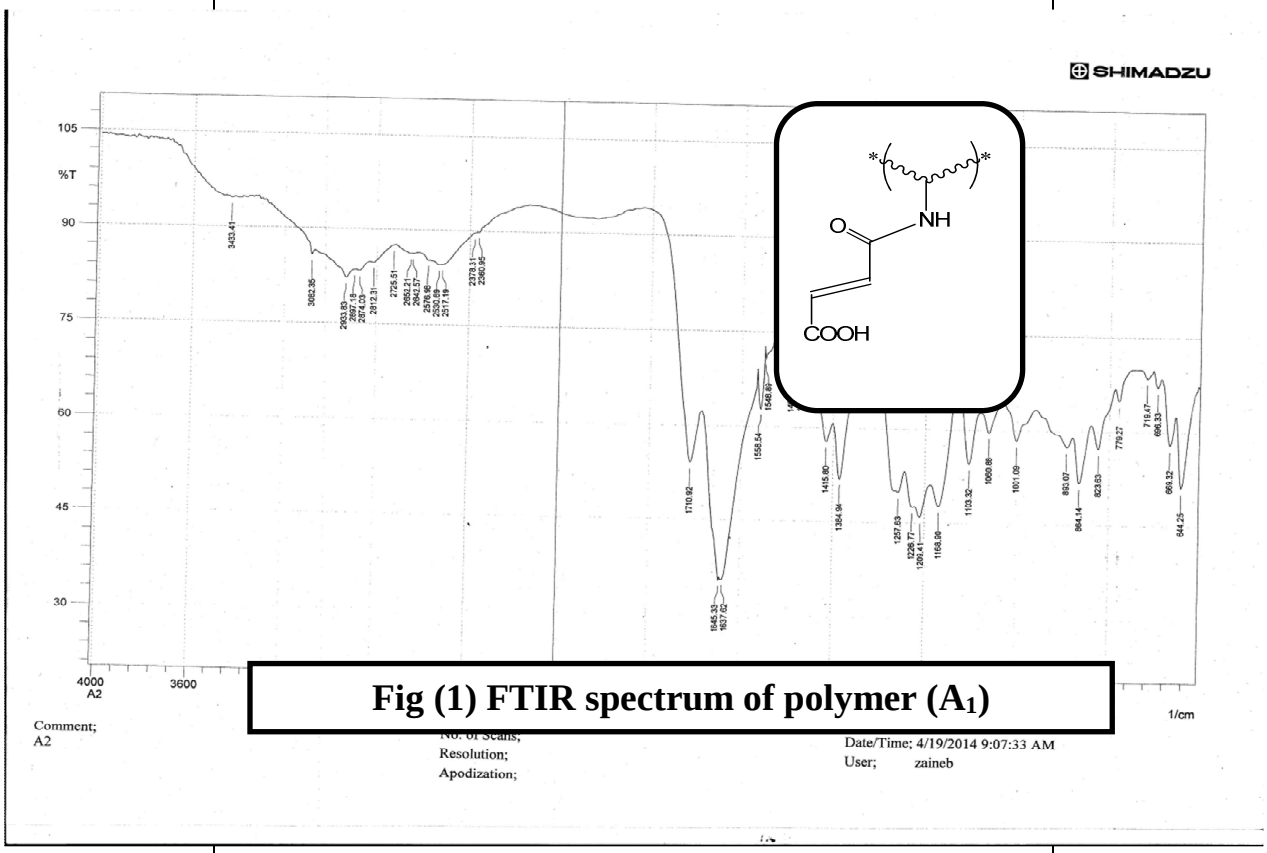


Fig (1) FTIR spectrum of polymer (A_1)

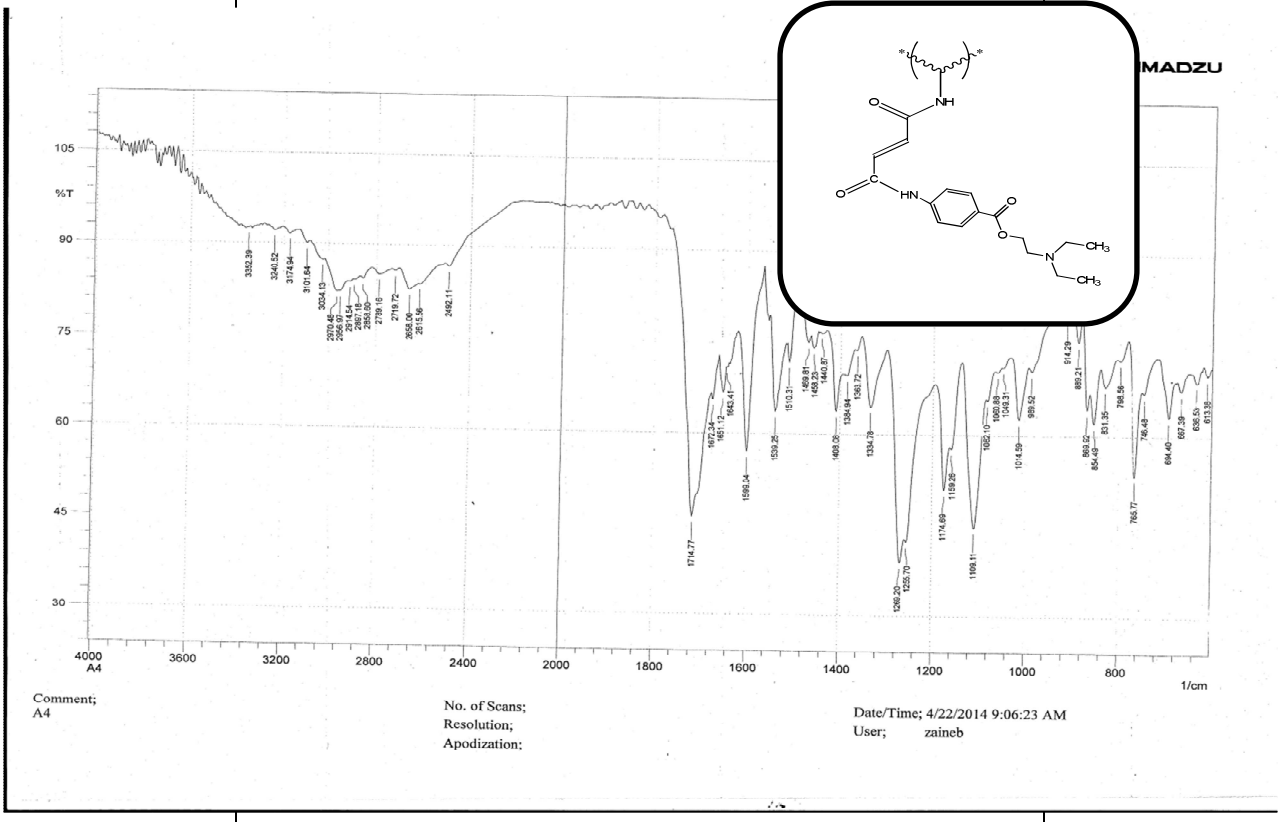


Fig (2) FTIR spectrum of polymer (A_2)

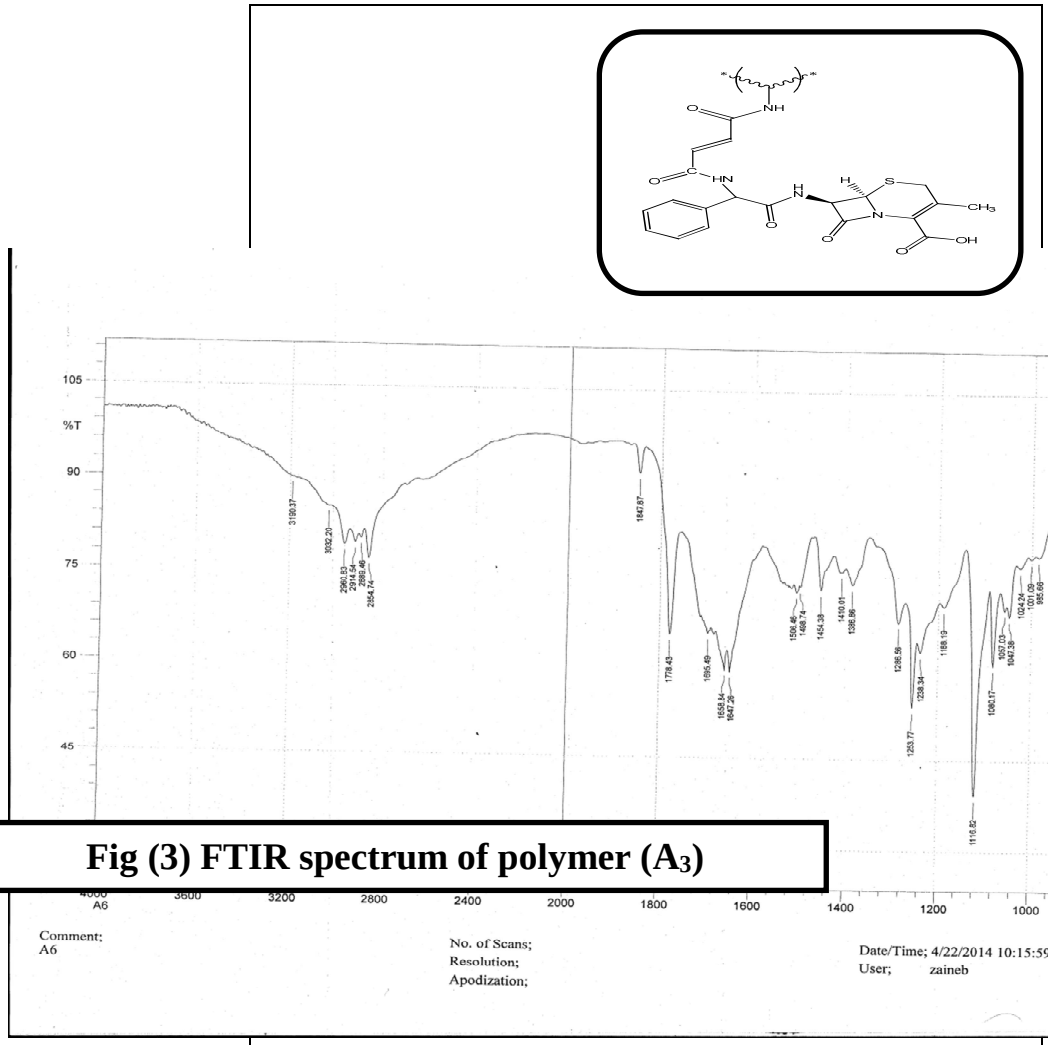


Fig (3) FTIR spectrum of polymer (A_3)

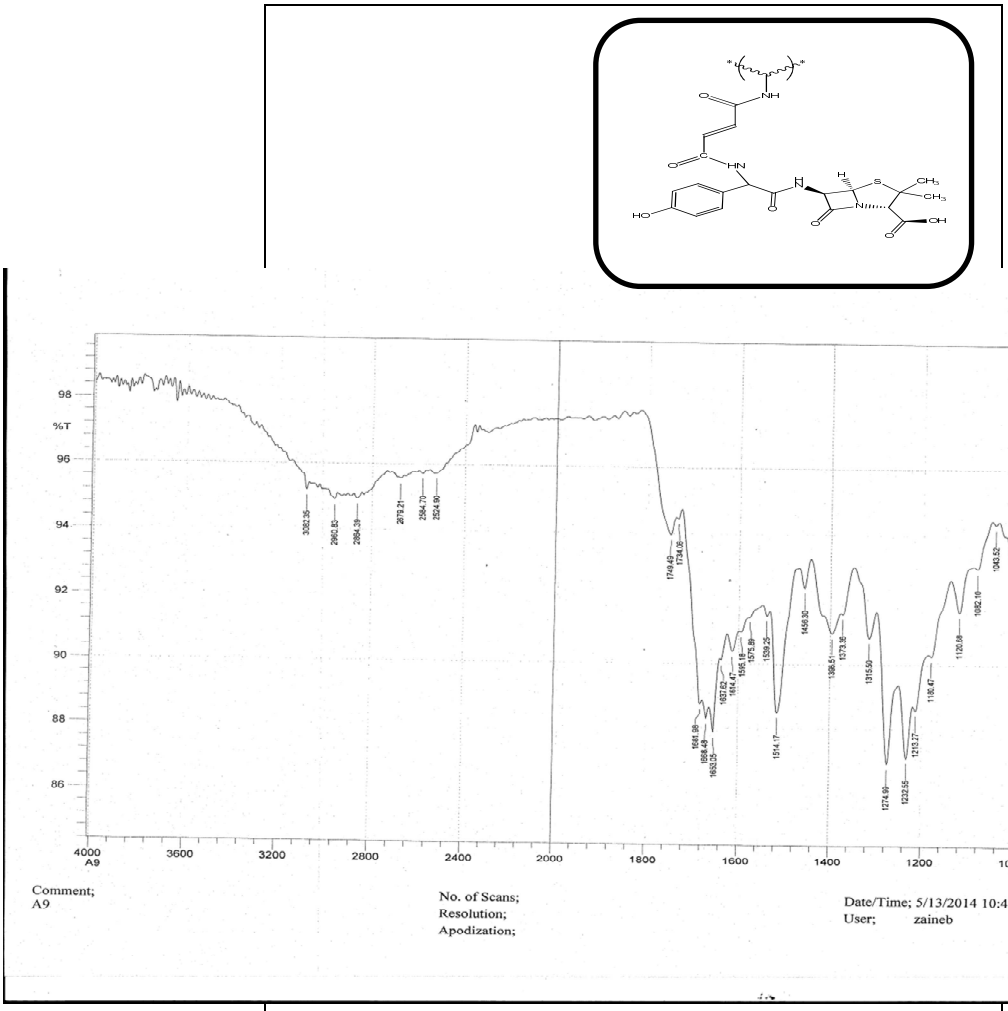


Fig (4) FTIR spectrum of polymer (A_4)

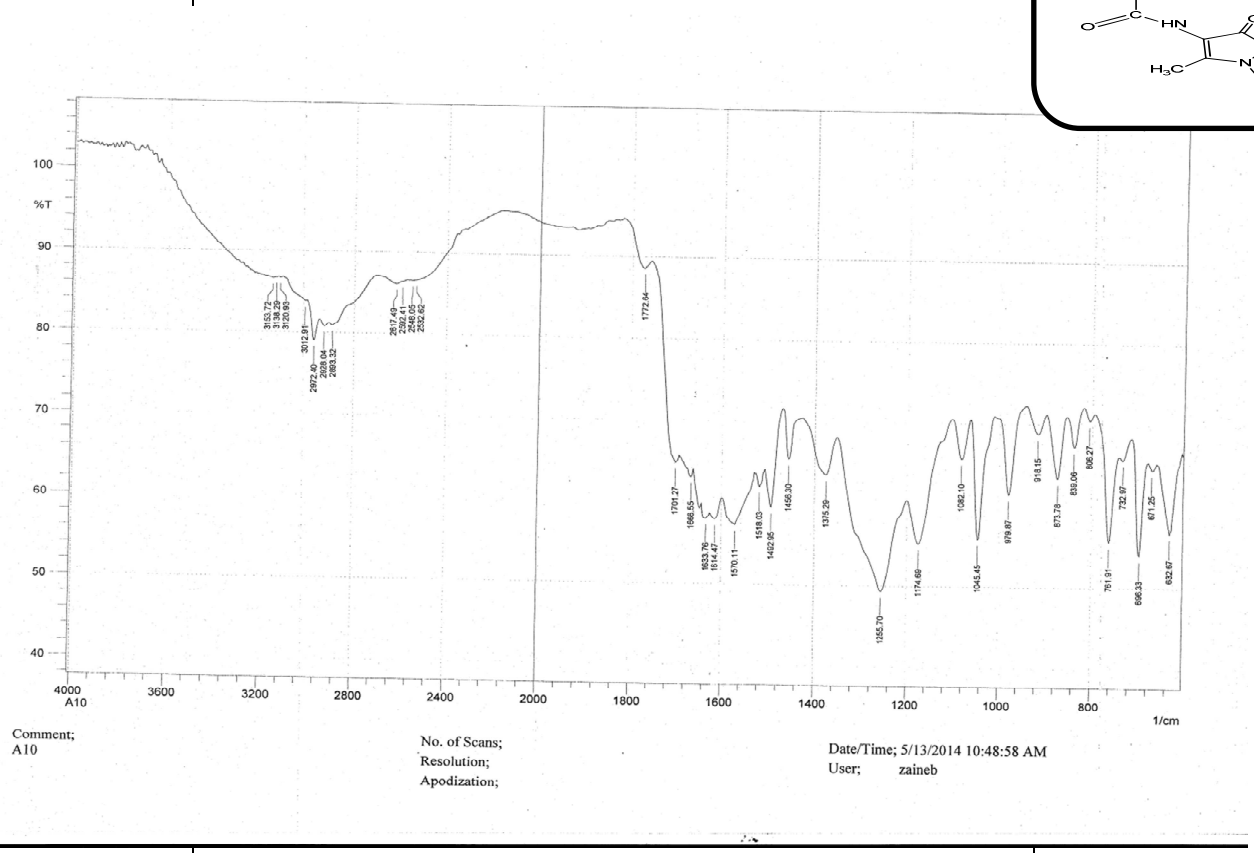


Fig (5) FTIR spectrum of polymer (A_5)

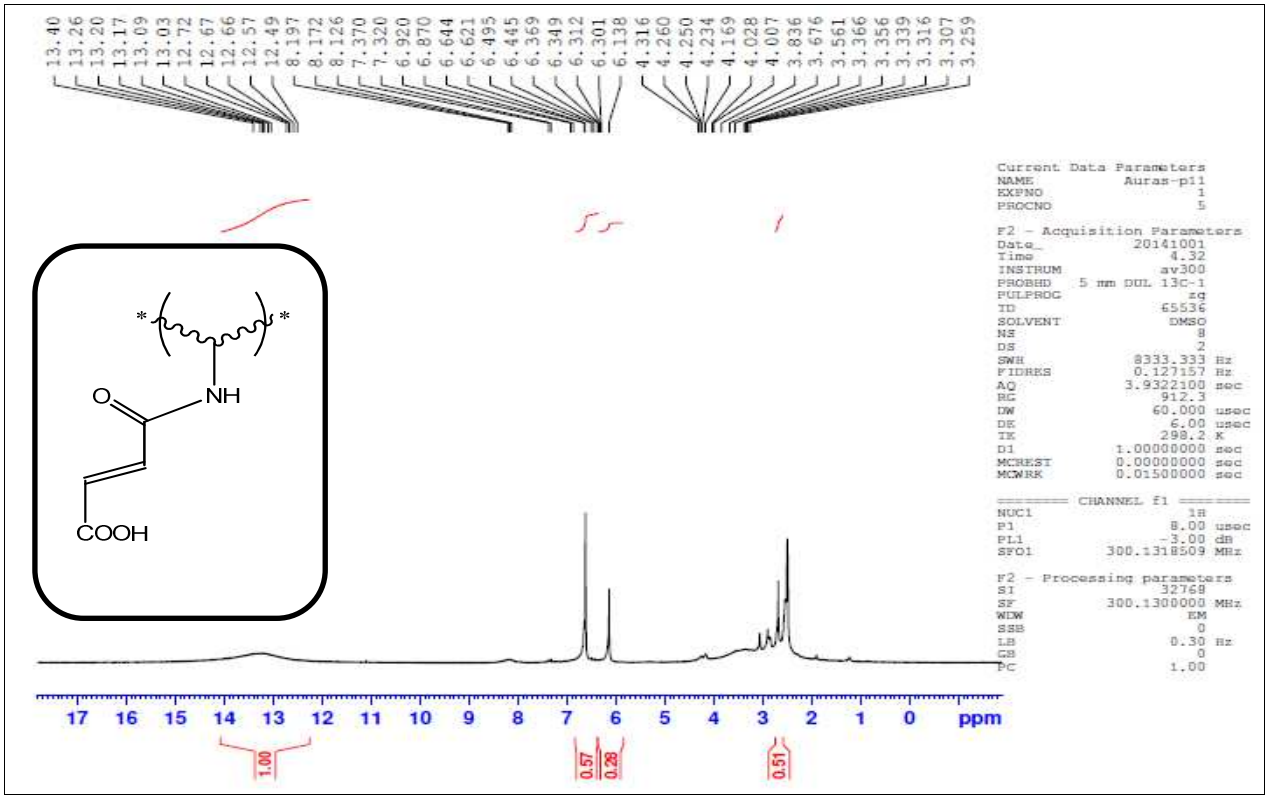


Fig (6) ^{1}H-NMR spectrum of polymer (A_1)

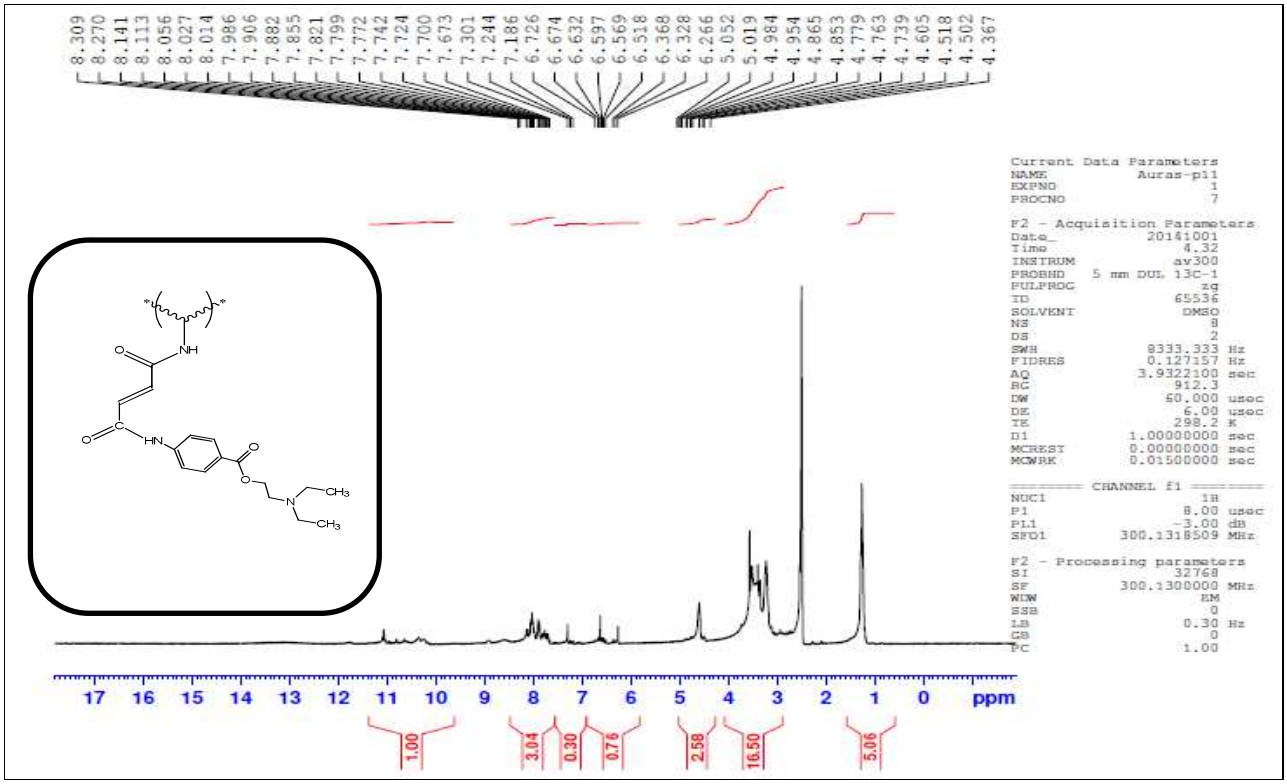


Fig (7) ^{1}H-NMR spectrum of polymer (A_2)

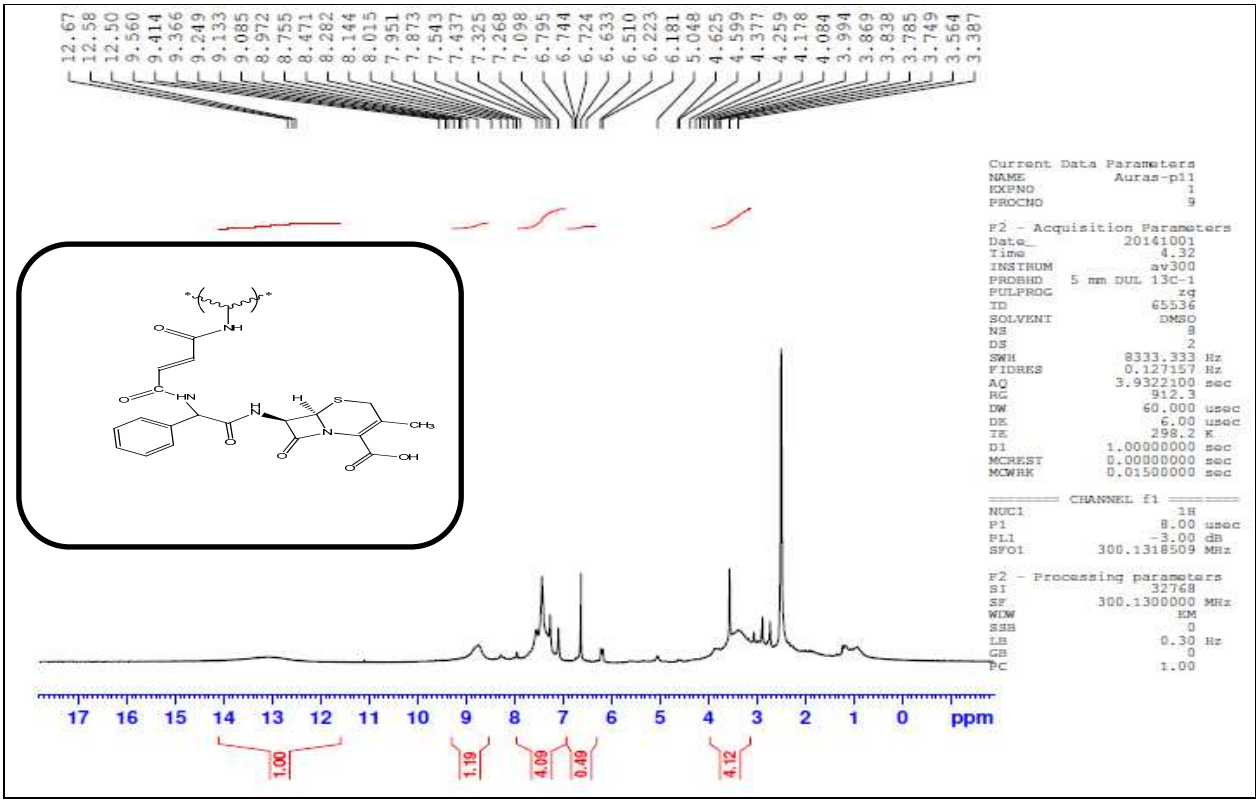


Fig (8) ^{1}H-NMR spectrum of polymer (A_3)

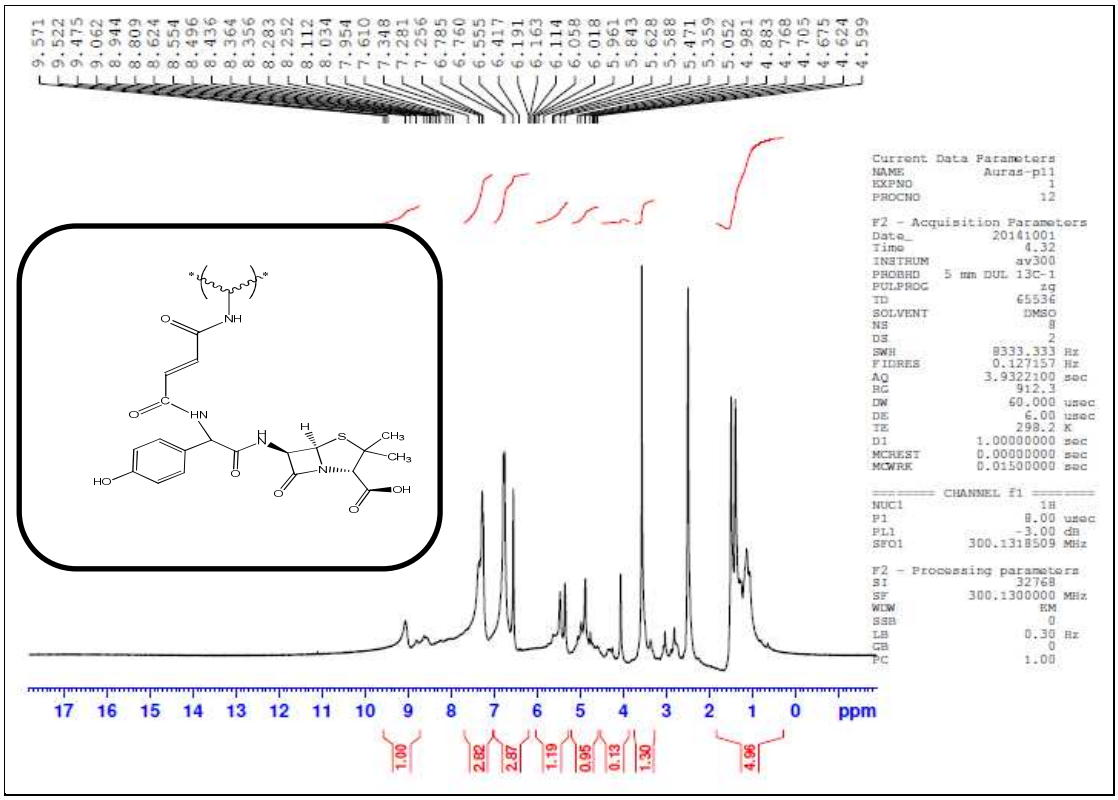


Fig (9) ^{1}H-NMR spectrum of polymer (A_4)

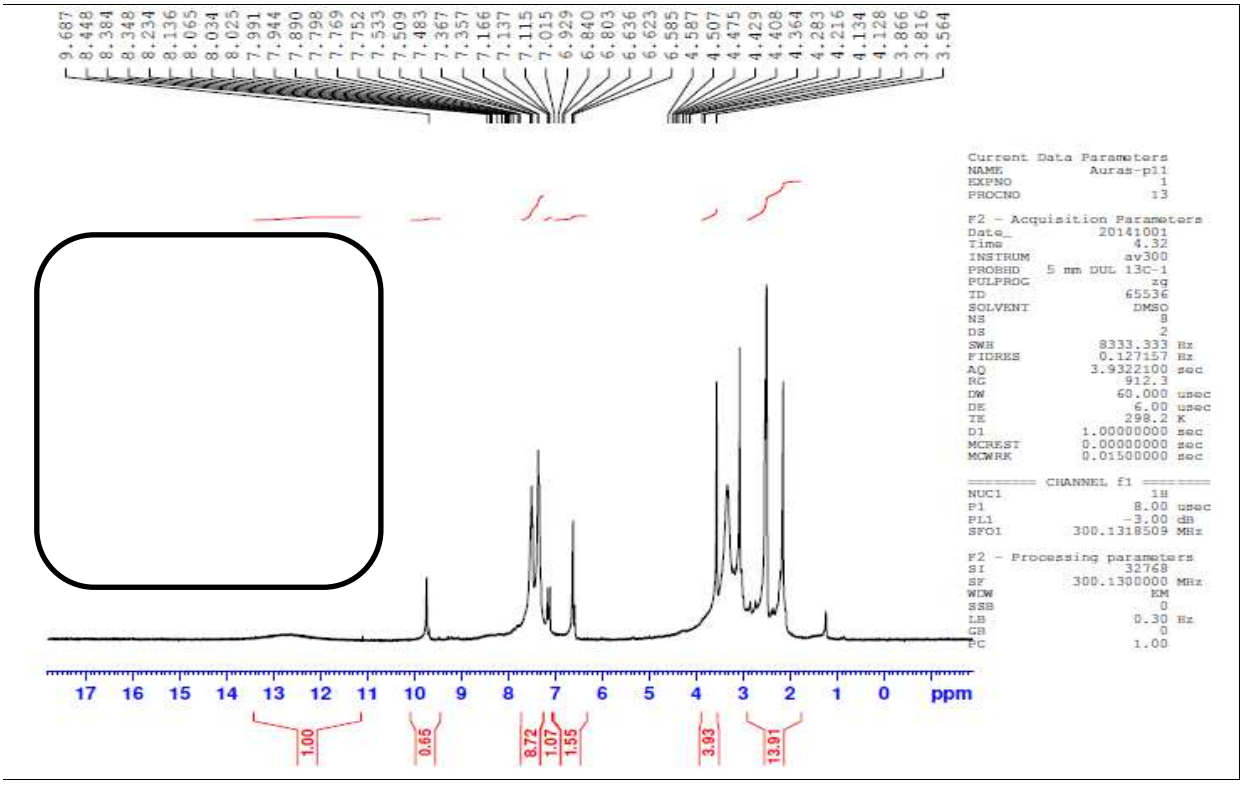


Fig (10) ^{1}H-NMR spectrum of polymer (A_5)

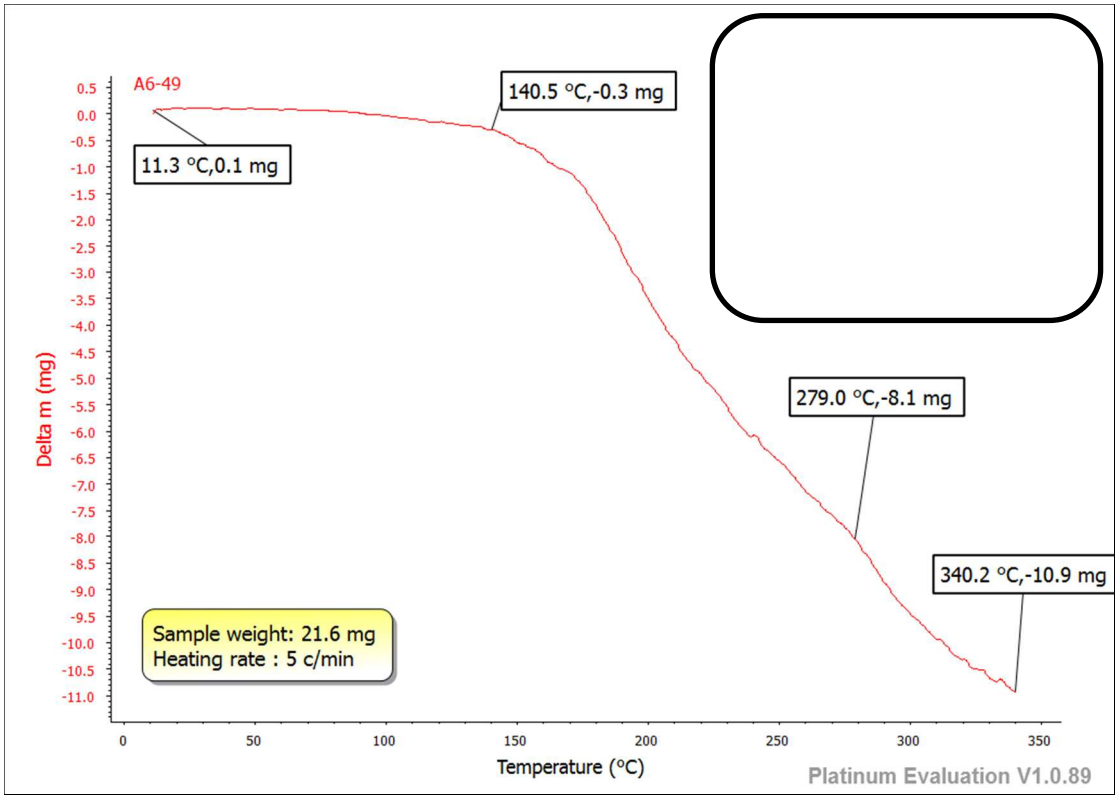


Fig (11A) TGAthermogram of compound (A_3)

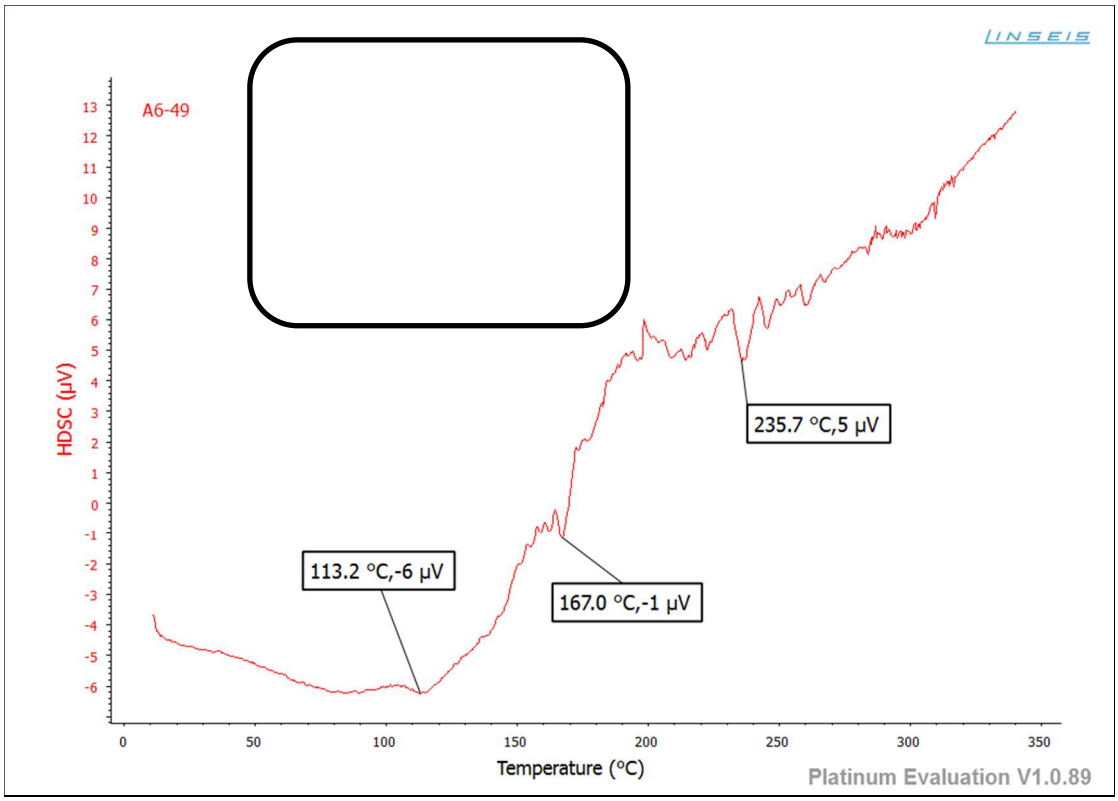


Fig (11B)DSCthermogram of compound (A_3)

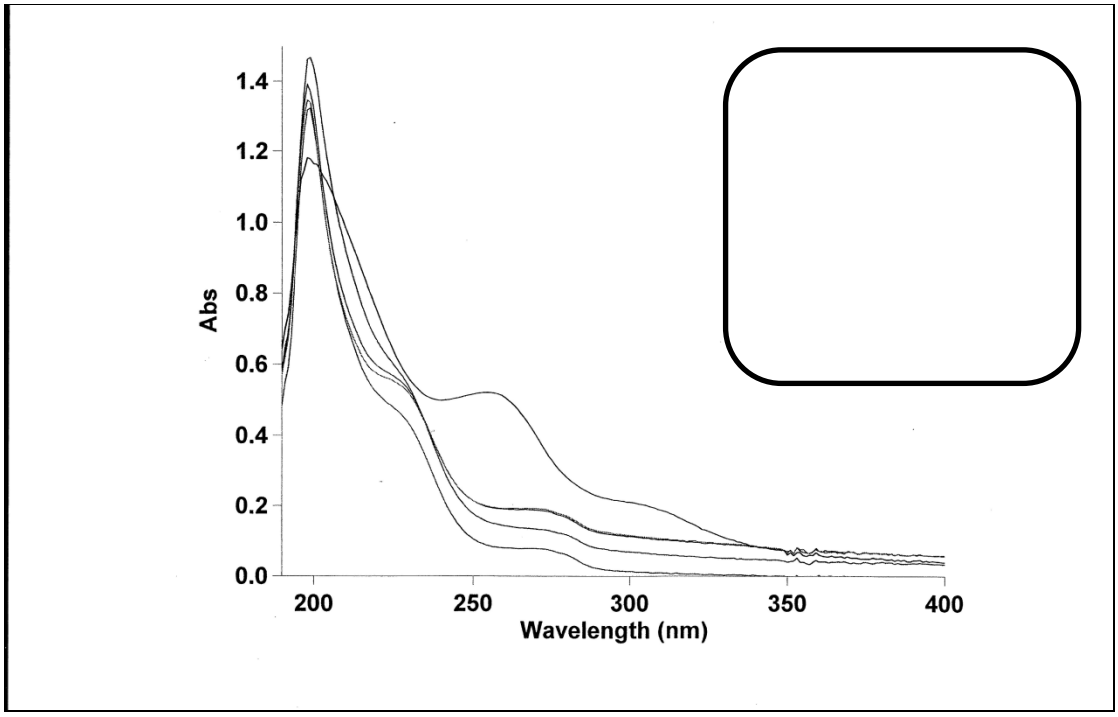


Fig (12A) UV. Spectra of prodrug(A_4) in pH 7.4

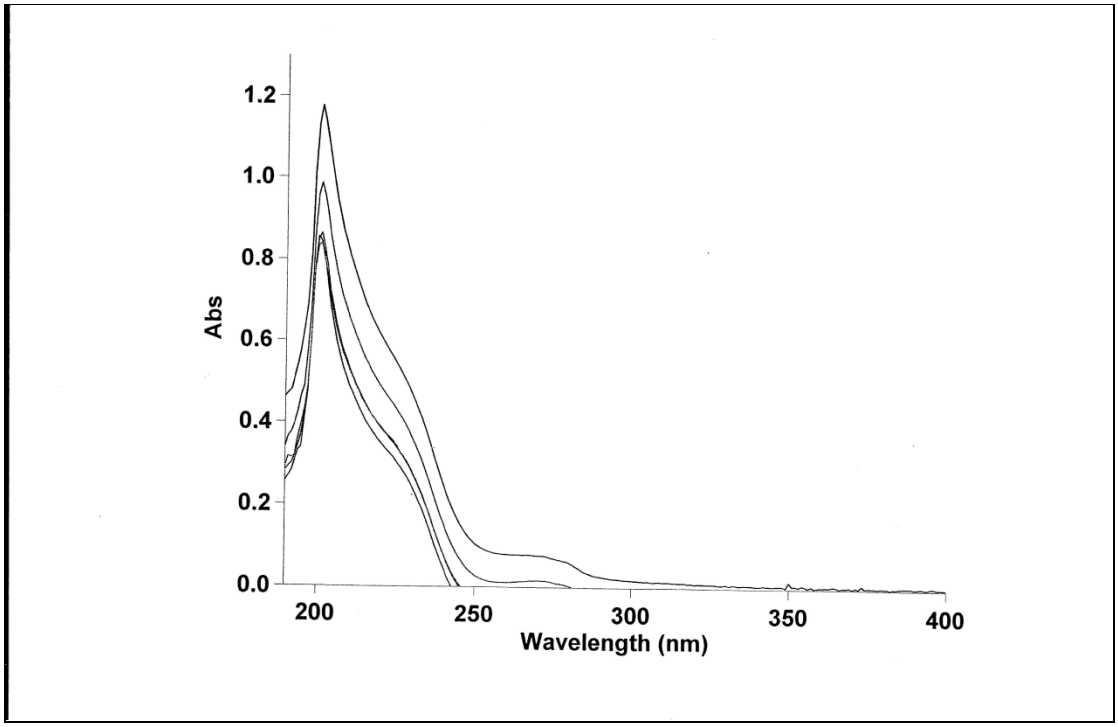


Fig (12B) UV. Spectra of prodrug(A_4) in pH 1.1

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