Preparation and study antibacterial activity of a new polymer

Farked Wahoody Salman Sami Wheed Radhi Amer Musa jodh
College of Science, Kufa University, Najf-Iraq.
farkedchemist@yahoo.com

Abstract

This search is including preparation of a new *N*-substitution maleimide of homopolymer and copolymer by five steps and study antibacterial activity.

1. preparation of azo compound by reaction of the sulfadiazine with 3-aminophenol to produced [A]
2. preparation of the amic acid by reaction between [A] compound with malic anhydride to produced[B]compound.
3. preparation of the monemer [*N*-substitution maleimide] by closed the ring and convert the [B]compound to [C] compound by access the acetic anhydride and sodium acetate.
4. preparation of the homopolymer by reaction[C] compound with AIBN as initiator and DMF as a solvent, to produced [D]compound.
5. preparation of the copolymer by reaction[C] compound with acrylamide in found AIBN as initiator and DMF as a solvent, to produced [C]compound.

FT-IR and ^{1}H NMR Spectra and C.H.N.S. analysis which reveals the confirmation of these compounds in good agreement.

The last step includes the study antibacterial activity of some prepared compounds against two types of bacteria: *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative).

Key words: sulfadiazine, maleimide, homopolymer, copolymer and antibacterial activity

الخلاصة

يتضمن هذا البحث تحضير بوليمرات الماليئماید المعوضة على ذرة N جديدة متجانس واخر مركب وبخمس خطوات ودراسة فعاليتها ضد البكتريا.

1-تحضير مركب الازو بتفاعل السلفادايازيين مع 3 – امينو فينول(مركب A)

2-تحضير الامك اسيد بتفاعل مركب (A) مع الما لك انهايدراید لينتج مركب (B)

3-تحضير المونمر الماليئماید المعوض بتحويل المركب B الى المركب (C) بواسطة الغلق بوجود الاسيتيك انهايدراید وصوديوم اسيتيت اللامائي

4-تحضير البوليمر المتجانس (D) بواسطة تفاعل المركب (C)مع AIBN كبادئ يولد الجذور الحرة وDMF كمذيب

5-تحضير البوليمر المركب من خلال التفاعل بين (C) و AIBNكبادئ يولد جذور حرة و DMF كمذيب والمثيل اكريليت.

المركبات المحضرة تم تشخيصها بواسطة مطيافية الأشعة تحت الحمراء والبعض منها شخصت بواسطة تحليل العناصر الدقيق كار بون،هيدروجين،نتروجين ومطيافية الرنين النووي المغناطيسي ألبروتوني وكذلك تم قياس درجات الانصهار للمركبات المحضرة.الخطوة الأخيرة تضمنت دراسة الفعالية لبعض المركبات المحضرة ضد نوعين من البكتريا هما:

1-*Staphylococcus aureus* (Gram positive) 2- *Pseudomonas aeruginosa* (Gram negative).

الكلمات المفتاحية: sulfadiazine, maleimide, homopolymer, copolymer and antibacterial activity

Introduction

Polymer is a chemical compound or mixture of compounds consisting of repeating structural units called monomer William(2008). Maleimide is the chemical compound with the formula $H_2C_2(CO)_2NH$ This unsaturated imide is an important building block in organic synthesis. The name is a contraction of maleic acid and imide, the -C(O)NHC(O)- functional group. Maleimides also describes a class of derivatives of the parent maleimide where the NH group is replaced with alkyl or aryl groups such as a methyl or phenyl, respectively.Maleimide and its derivatives are prepared from maleic anhydride by treatment with amines followed by dehydration IUPAC, (1997) .

N-substituted maleimides can be obtained by treatment of maleic anhydride with primary amines in an inert solvent. N-phenylmaleimide has also been prepared by mixing maleic

anhydride and aniline in ethyl ether at room temperature, and by treating the maleanilic acid with a mixture of acetic anhydride and anhydrous sodium acetate Sperling(2006).Maleimides have been polymerized by addition polymerization with either free radical or anionic initiation. Anionic polymerization of N-phenylmaleimide.(European Chemicals Agency,(2012), Hagiwara,(1990) The imidization reaction of maleic anhydride is a two-step condensation reaction. In the first step, maleic anhydride is dissolved in a good solvent which then reacts with the primary amine to produce an intermediate product, N- substituted maleamic acid. In the second step which is performed at an elevated temperature in a mixture of sodium acetate and acetic anhydride, the intermediate product undergoes dehydration and ring closure to produce N-substituted maleimide IUPAC, (1997).

Experimental

All chemicals used were supplied from Merck , BDH and Fluke chemicals company .Melting points were recorded using Electro thermal melting point apparatus , UK. The FT-IR spectra were recorded using Fourier transform infrared SHIMADZU FT.IR-8400S infrared spectrophotometer by KBr disc, University of Kufa. The elemental analysis were recorded using E.A.G.E.R.-100, Carlo Erba, Italy, measurements were made at the Department of Chemistry, Kufa University. Thin layer chromatography (TLC) was performed on aluminum plates and coated with layer of silica gel, compounds were detected by iodine vapor.^{1}H NMR were recorded on Fourier transformation Bruker spectrometer, operating at(400 MHz) with (DMSO-d_6), measurement were made at the department of chemistry, Kashan University, Iran.

first step(preparation of a compound)(Azo)(A compound)

3- amino phenol (0.05mole)(5.45gm) was dissolved in (3ml) of concentrated hydrochloric acid and (15 ml) of distilled water. The mixture was cooled at (0-5 ^{0}C) in ice-water bath ,Then a solution of sodium nitrite (0.05mole)(3.45gm) was dissolved in (5 ml) of distilled water then it will be cooled at (0-5 ^{0}C). This solution was added a drop wise to the mixture with stirring at the same temperature. The diazonum salt solution was added portion wise to solution of (0.05mol)(12.5gm) sulfadiazine in distilled water with sodium hydroxide (1.5 gm) dissolved in (100ml) distilled water. The PH was maintained with (9-10) and temperature at (0-5^{0}C).The mixture was stirred for 30 mint. The mixture was left over night. The product was precipitated and filtered, washed well with sodium bicarbonate and distilled water and re-crystallized from ethanol(black brown)(yield 95%).

(A)compound:4-amino-3-((3-hydroxyphenyl)diazenyl)-N-(pyrimidin-2-yl) benzenesulfonamide

Second step (preparation of amic acid)(B compound)

A solution of maleic anhydride (1.64gm , 0.016 mol) in DMF was gradually added over aperiod of 10 minutes to a well-stirred solution of (A) compound (6.2gm, 0.016mol) inDMF. The mixture was stirred for 2 hours at room temperature. The resulting solution was poured into a large amount of crushed ice to precipitate crude (B). The crude was filtered, dried and then recrystallized from ethanol to obtain pure (B) in 85% yield (yellow brown) .(B) compound : 4-(2-(3-hydroxyphenyl)diazenyl)-4-(N-pyrimidin-2-ylsulfamoyl)phenylamino)-4-oxobut-2-enoic acid

Third step (preparation of monomer) (C compound)

A mixture of (2gm) (0.004mole)B compound , 0.6g sodium acetate and 200 mlacetic anhydride were stirred for 5 hours at 55-60°C. Cooled reactionmixture was pouredinto large amount of crushed ice, brownmass obtained of (C) compound was filtered anddried at 60-70°C and thenrecrystallized from methanol obtaining the product in a 75% yield (light brown

(C) compound : 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-((3-hydroxyphenyl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide

Fourth step (preparation of homopolymer) (D compound)

(C) compound(0.001mol,0.5gm),were carried outwith AIBN as afreeradical initiator in THF and DMF (70 , 30 ml), in a round bottom flask. Reactionmixture was refluxed at 65°C for 48 hour under nitrogen . The homopolymerwas isolated byprecipitation in methanol containing water. The precipitatedhomopolymer was washed with methanol several times and dried (yield 62%)(brown) .(D) compound : poly-4-(3,4-dimethyl-2,5-dioxopyrrolidin-1-yl)-3-((3-hydroxyphenyl)diazenyl)-N-(pyrimidin-2yl)benzenesulfonamide

Fifth step (preparation of copolymer)(E compound)

(C)compound(0.001mol,0.5gm) and acrylamide(0.3gm),were carried out with AIBN as a free radical initiator in THF and DMF (70 , 30 ml), in a round bottom flask. Reaction mixture was refluxed at 65°C for 48 h urunder nitrogen . The copolymer was isolated byprecipitation in methanol containing water. The precipitated copolymer was washed with methanol several times and dried(yield 71%)(yellow brown)

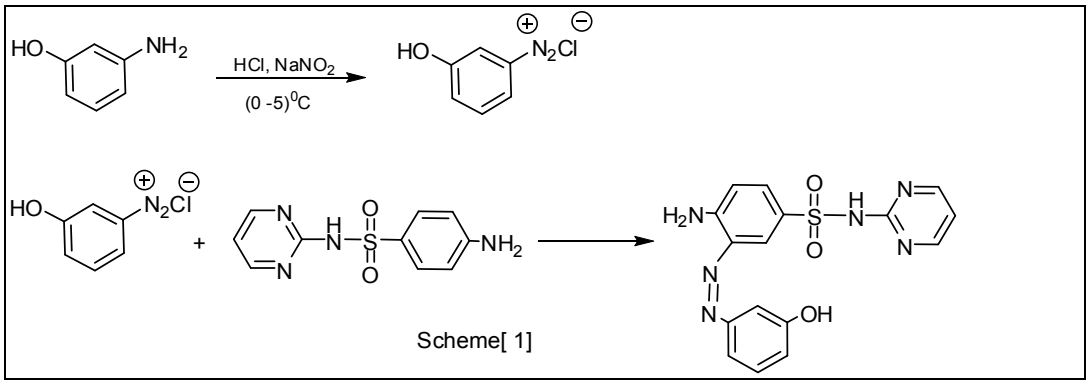
(E)compound:copoly-3-(1-(2-((3-hydroxyphenyl)diazenyl)-4-(N-pyrimidin-2-ylsulfamoyl)phenyl)-4-methyl-2,5-dioxopyrrolidin-3-yl)-2-methylpropanamide.

Antibacterial activity

Compounds[D and E] were used for their antibacterial activity against the *Staphylococcus aureous* (Gram-positive) and *Pseudomonase aeroginosa* (Gram-negative) by Well diffusion method . Each isolated bacteria was inoculated on to the Muller-Hinton Agar (MHA) [sterilize in autoclave] by dipping a cotton swab in to the suspension and streaking over the surface of the agar plates. Then, in the solidified medium, four holes were made (6 mm). These holes were filled with (0.5 ml) of the prepared compounds (15,30 mg) of the compound dissolved in 1ml of DMSO solvent). These plates were incubated at 37 ^{0}C and measured of zone inhibition after 24 hours.

Synthesis and identification of azo compound [A]

3-Aminophenol was converted to 3-Aminophenol diazonium chloride by reaction with concentration hydrochloric acid and sodium nitrite. Diazonium salt was directly introduced in a coupling reaction with sulfadiazine to produce [A]. Scheme [1].



Scheme[1]

The [C.H.N.S.] analysis of synthesized compound [A] was accepted agreement with the calculated percentage of elements showed in Table [1].The FT-IR spectra of this compound showed appearance of stretching vibration band at 3417 cm^{-1} due to the stretching vibration of (OH) group, with remaining of two absorption bands at 3300,3223 cm^{-1} of the asymmetric

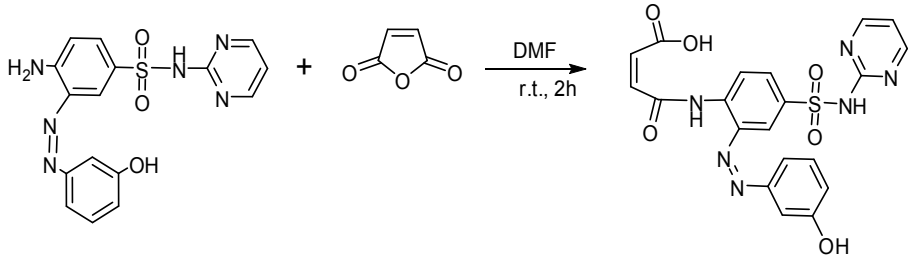
and symmetric stretching vibration of (-NH_2)and absorption bands at 3458 $cm^{-1}$of the symmetric stretching vibration of (-NH). The azo stretching vibration of (N=N) were appeared between 1436cm^{-1} of compound [A]. All these absorption bands are another good evidence to preparation compound [A]. Other characteristic bands in Table [1].

Table [1] [C.H.N.S.] analysis data and physical properties of compound [A]

Com. NO.	C.H.N.S. data Calculated found				M.P. ^0C	Yield %	Infrared data (V,cm^{-1}) (KBr disc)
	C%	H%	S%	N%			
A	(51.88) 51.575	(3.81) 3.721	(8.66) 8.575	(22.69) 22.441	Above 300	95	3417(O-H), 3358(N-H), 3300(N-H_2), 3043(C-H), 1500(C=N), 1355(SO_2), 802(C-S)

Synthesis and identification of amic acid [B]

Malic anhydride was reacted with[A] compound to produced [B] compound by using DMF as a solvent at room temperature. Scheme [2].



Scheme [2]

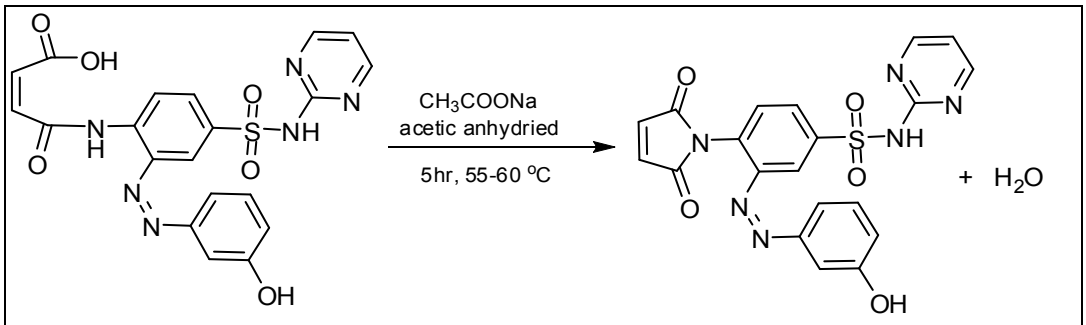
The [C.H.N.S.] analysis of synthesized compound [B] was accepted agreement with the calculated percentage of elements showed in Table[2]. The FT-IR spectra of this compound showed remaining of stretching vibration band at 3425 cm^{-1} due to the stretching vibration of (OH) group of phenol and appearance of stretching vibration band at 3361 cm^{-1} due to the stretching vibration of (OH) group of carboxylic acid, with remaining of absorption band at 3255 cm^{-1} of the symmetric stretching vibration of (-NH). Appearance absorption band at 1714 cm^{-1} due to stretching vibration of (C=O) group of carboxylic acid and absorption band at 1585 cm^{-1} due to stretching vibration of (C=O) of amid group. All these absorption bands are another good evidence to preparation compound [B]. Other characteristic bands in Table [2].

Table [2] [C.H.N.S.] analysis data and physical properties of compound [B]

Com. NO.	C.H.N. data Calculated found				M.P. ^{0}C	Yield %	R_f	Infrared data (V,cm^{-1}) (KBr disc)
	C%	H%	S%	N%				
B	(51.28) 51.131	(3.44) 3.301	(6.85) 6.752	(17.94) 17.713	Above 300	85	0.69	3425 (O-H), 3255 (N-H), 2939(C-H) $_{Al}$, 1714 (C=O) $_{Car}$, 1585 (C=O)$_{Ami}$, 1327(SO_2)

Synthesis and identification of monemer [C]

Amic acid was converted to *N*-substitution maleimid (monomer) by using a mixture of acetic anhydride and anhydrous sodium acetate at (55-60)^{o}C for 5 hr. Scheme [3].



Scheme [3]

The [C.H.N.S.] analysis of synthesized compound [C] was accepted agreement with the calculated percentage of elements showed in Table [3]. The FT-IR spectra of this compound showed remaining of stretching vibration band at 3431 cm^{-1} due to the stretching vibration of (OH) group of phenol and stretching vibration band at 3419cm^{-1} due to the stretching vibration of (-NH) group, with remaining absorption band at 1722 cm^{-1} of stretching vibration of carbonyl groups. Appearance absorption band at 1593 cm^{-1} due to stretching vibration of (C=C) vinyl group. All these absorption bands are another good evidence to preparation compound [C]. Other characteristic bands in Table [3].

Table [3] [C.H.N.S.] analysis data and physical properties of compound [C]

Com. NO.	C.H.N.S. data Calculated found				M.P. ^{0}C	Yield %	R_f	Infrared data (V,cm^{-1}) (KBr disc)
	C%	H%	S%	N%				
C	(53.33) 53.328	(3.13) 3.117	(7.129) 7.128	(18.66) 18.575	Above 300	75	0.72	3431(O-H), 3419 (N-H), 3109(C-H) $_{Ar}$, 1722 (C=O) , 1593 (C=C), 1371(SO_2)

^{1}H NMR spectrum (δ ppm), Figure [1] of (C) showed the following characteristic of chemical signals (DMSO-d_6) as a solvent**, ((1H), (-NH)$_{sulfonamide}$ 11.1), ((1H) (OH)$_{phenol}$ 9.2) , ((10H) (Ar-H)) 6.8-8.4), ((2H) (CO-CH=CH-CO) 6.00).**

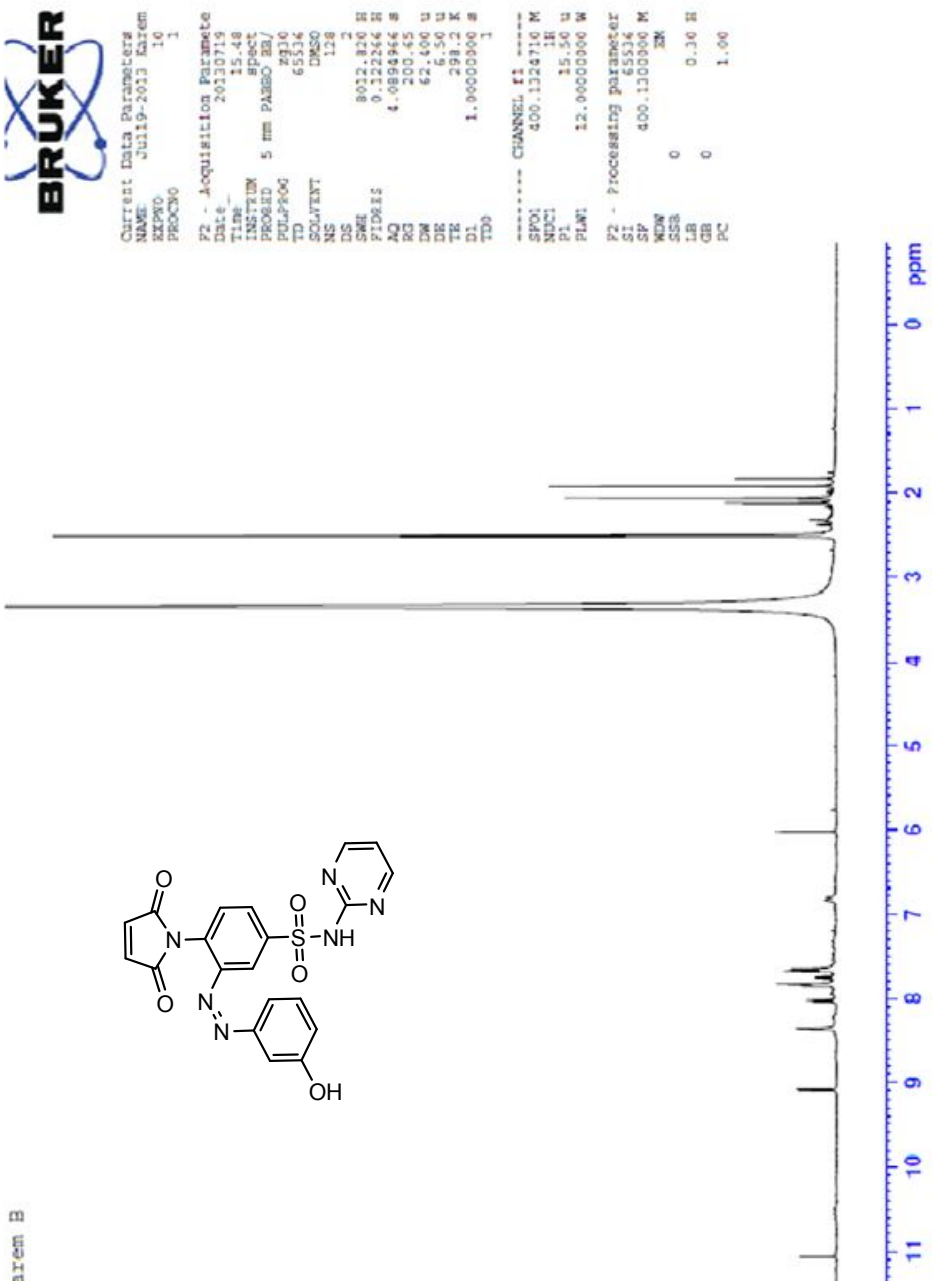
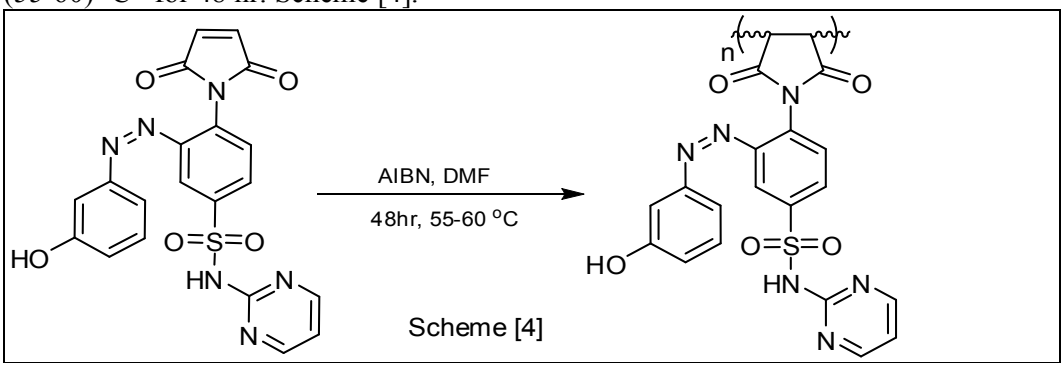


Figure [1] ^{1}H NMR spectrum of compound [C]

Synthesis and identification of homopolymer [D]

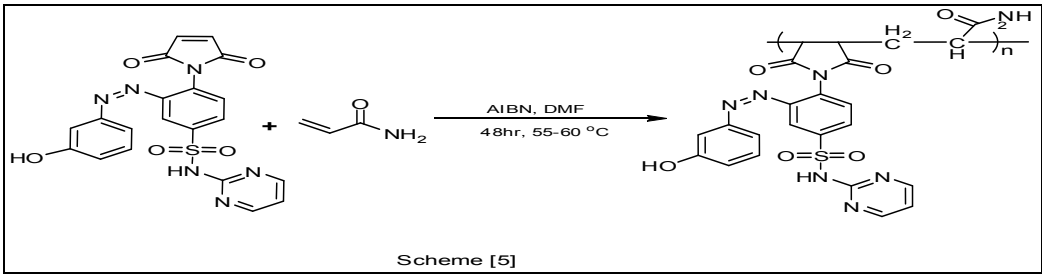
Polymerized of monomer [C] compound by AIBN as initiator with DMF as a solvent at (55-60) ^{o}C for 48 hr. Scheme [4].



Scheme [4]

Synthesis and identification of Copolymer [E]

Polymerized of monomer [C] compound with acryl amid by AIBN as initiator with DMF as a solvent at (55-60) ^{o}C for 48 hr. Scheme [5].



Scheme [5]

The FT-IR spectra of compound [D] showed remaining of stretching vibration band at 3408 cm^{-1} due to the stretching vibration of (OH) group of phenol and stretching vibration band at 3408cm^{-1} due to the stretching vibration of (-NH) group, with remaining absorption band at 1702 cm^{-1} of stretching vibration of carbonyl groups. Disappearance absorption band at 1593 cm^{-1} due to stretching vibration of (C=C) vinyl group. The FT-IR spectra of compound [E] showed remaining of stretching vibration band at 3427 cm^{-1} due to the stretching vibration of (OH) group of phenol and stretching vibration band at 3427cm^{-1} due to the stretching vibration of (-NH) and(-NH_2) groups, with remaining absorption band at 1687cm^{-1} of stretching vibration of carbonyl groups. Disappearance absorption band at 1593 cm^{-1} due to stretching vibration of (C=C) vinyl group. All these absorption bands are another good evidence to preparation compound [D and E]. Other characteristic bands in Table [4].

Table [4] some physical properties of compound [D and E]

Com. NO.	M.P. ^0C	Yield%	Infrared data (V,cm^{-1}) (KBr disc)
D	Above 300	73	3408(O-H), 3408 (N-H),3408 ($N-H_2$), 3109(C-H) $_{Ar}$, 2951(C-H) $_{Al}$ 1708 (C=O) , 1327(SO_2)
E	Above 300	85	3600(O-H), 3600 (N-H),3600 ($N-H_2$), 3111(C-H) $_{Ar}$, 2941(C-H) $_{Al}$ 1759 (C=O) , 1330(SO_2)

^{1}H NMR spectrum (δ ppm), Figure [2] of (D) showed the following characteristic of chemical signals (DMSO-d_6) as a solvent**,**
((1H), (-NH)$_{sulfonamide}$ 11.3), ((1H) (OH) $_{phenol}$ 9.1) , ((10H) (Ar-H)) 6.8-8.5), ((2H) (CO-CH-CH-CO) 2.8).

^{1}H NMR spectrum (δ ppm), Figure [3] of (E) showed the following characteristic of chemical signals (DMSO-d_6) as a solvent**,**
((1H), (-NH)$_{sulfonamide}$ 11.2), ((1H) (OH) $_{phenol}$ 9.3) , ((2H), (-NH_2)$_{acryl\ amide}$ 7.1), ((10H) (Ar-H)) 6.8-8.3), ((2H) (CO-CH-CH-CO) 2.9) ,((1H) (NH_2-CO-CH-) 1.8).

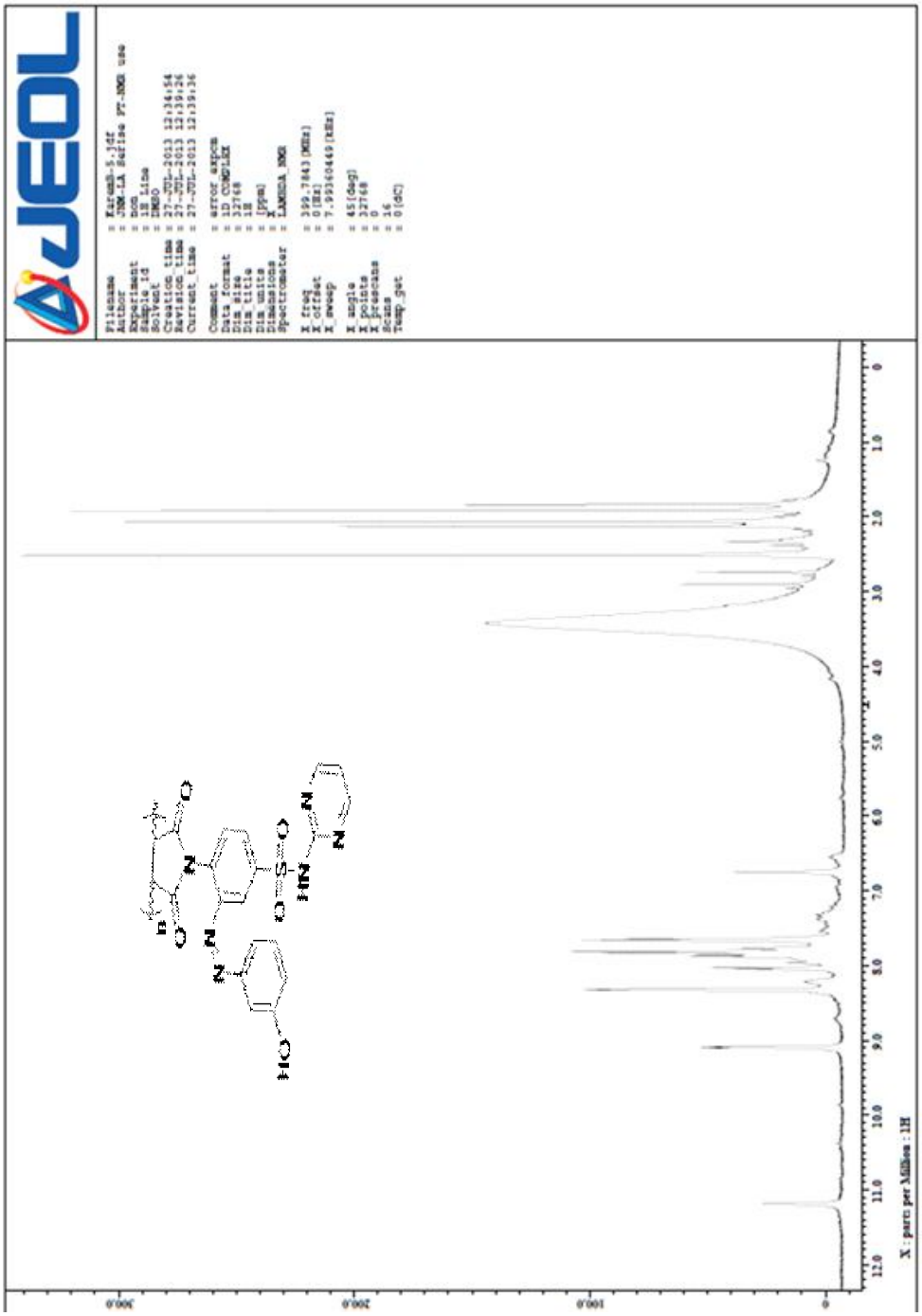


Figure [2] ^{1}H NMR spectrum of compound [D]

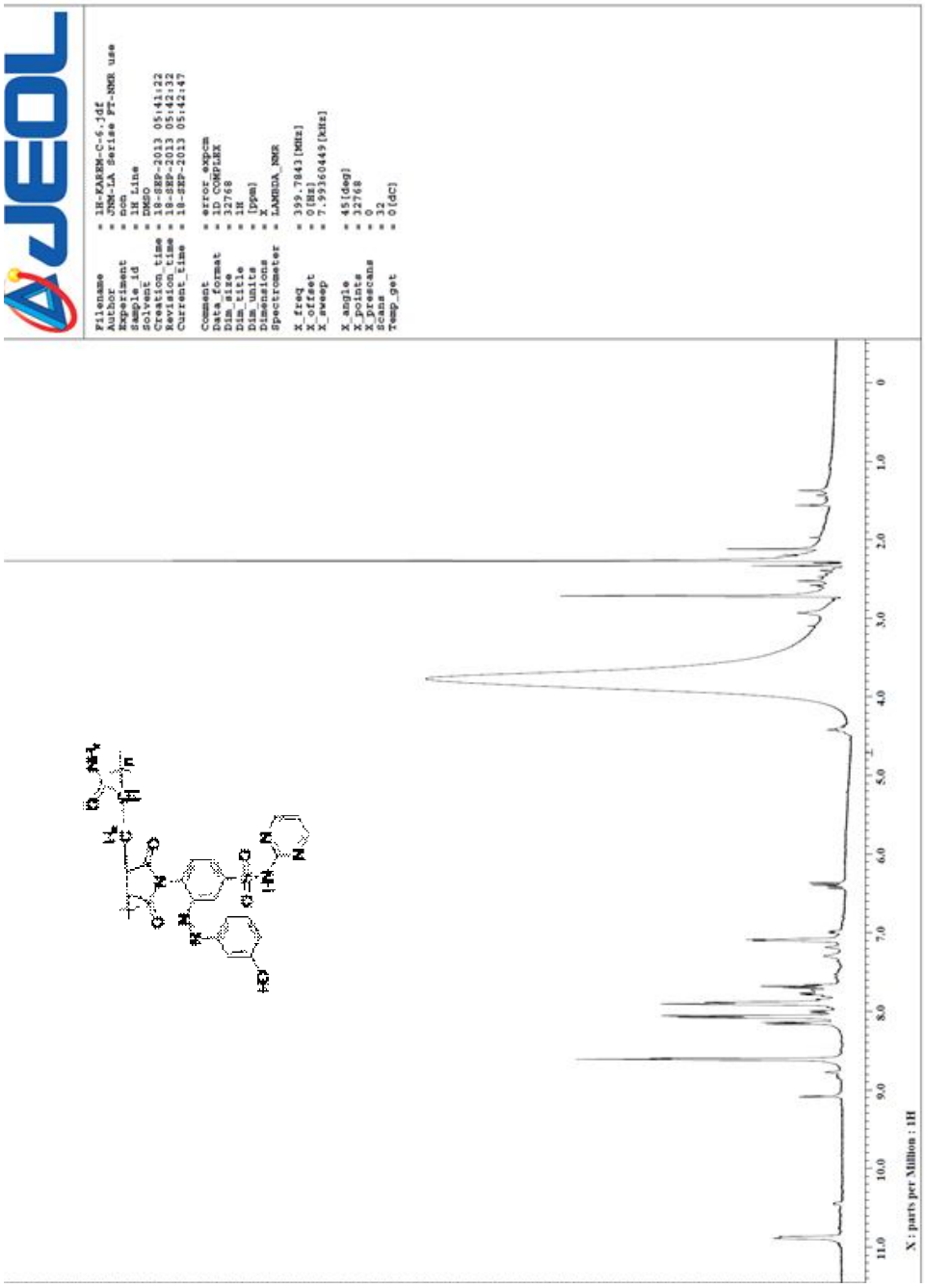


Figure [3] ^{1}H NMR spectrum of compound [E]

Antibacterial activity

The prepared [D] and [E] compounds were examined for antibacterial activity against *Staphylococcus aureous* (Gram-positive)and *Pseudomonase aeroginosa* (Gram-negative) by well diffusion method in Mueller-Hinton agar medium. After 24 hours were measured for zone of inhibition around each disc. The test results presented in Table [5] showed that [D] and [E] compounds was exhibited highly active against these are bacterial with increase concentration depend on the experimental [Morad(2007), Wadher(2009)] .

Table [5] Antibacterial activity of polymer synthesized

polymer Code	Sample Weight (mg)	*Staphylococcus aureous* (Gram-positive)	*Pseudomonase aeroginosa* (Gram-negative)
D2	15	+	+
D1	25	+++	+++
E2	15	+++	+++
E1	25	+++	+++

Key of symbols:
Highly active = +++ (inhibition zone > 15 mm)
Moderately active = ++ (inhibition zone 11-15 mm)
Slightly active = + (inhibition zone 5-10 mm)

References

EuropeanChemicals Agency, 2012 "Guidance for monomers and polymers"

Hagiwara, T. T. Shimizu, T. Uda, H. Hamana and T. Narita, J.1990 *Polvm. Sci.. Part A: Polvm. Chem.*. 28, 185-92

IUPAC, N. E. "Compendium of Chemical Terminology", 2nd ed. (the "Gold Book") (1997). Online corrected version: "macromolecule (polymer molecule) (2006)

Morad F.M., Ajaily M. And Gweirif S.B.; 2007 *Journal of science and Its applications*;1(1),P.72-78

Sperling, L. H. (Leslie Howard) 2006 "Introduction to physical polymer science". Hoboken, N.J.: Wiley. p. 10.

Wadher S.J., Pulanik M.P., Karande N. A. and Yeole P.G.; 2009.Int. J. pharm Tech Res;1(1),p.22-33

William B. Jensen Ask the Historian: 2008 "The origin of the polymer concept," Journal of Chemical Education, 88 : 624-625. Available on-line at: University of California.edu.