Synthesis, Characterization and Biological Activity Evaluation of Bis-Methylene-di-(2-Amino Benzoic Acid) Derivatives Grafting with Polyethanol

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Summary: A series of new heterocyclic aromatic derivatives compounds were synthesized by reacted of Bis-methylene-di-(2-aminobenzoyl chloride) with different chloride moieties such as piperidine-1-sulfonylchloride, Pryidine-3-sulfonylchloride, 4,4'-(diazene-1,2-diyl)dibenzoyl-chloride and chloramphenicol respectively, to afforded a derivatives compound containing primary amine group [A-A4]. These derivative compounds [A-A4] were reacted with polyethanol using cold-warm esterification to synthesize of new graft polymers [B1-B4]. The structures of products were conforming by FT-IR and ^{1}H-NMR spectroscopy. XRD-Diffraction of grafted polymer showed compound [B1 and B3] were crystal and semi-crystal respectively. As well as, derivatives were tested by swelling test. The results of swelling showed a higher range between 50-150 % at 72 hrs. These compounds [A-A4] and [B1-B4] have been assayed of biological activity against Escherichia coli G+ve as well as Staphylococcus aurous G-ve microorganisms.

Key words: bis-methylene-di-(2-aminobenzoic acid), pipyridine-1-sulphonyl chloride, pyridine-3-methylsulphonylchloride, chloramphenicol, antimicrobial.

Introduction

Aromatic and heterocyclic aromatic compounds are thought about the backbone of the synthesis of most crucial industrial and pharmaceutical compounds, especially those modified with polymers [1]. The renewed interest in natural polymer resins is a result of environmental concerns such as recycling and environmental safety [2]. Unlike petroleum-derived thermoplastics, which are believed to be harmful and non-biodegradable [3]. Microorganisms act on biodegradable polymers to break them down and release carbon dioxide and water [4]. The most widely used polymer in adhesive products and the production of other polymers is polyethanol, which is sometimes referred to as polyethylene (polyvinyl alcohol) [5]. Polyethanol is a generally available, low-cost polymer that looks like a white powder [6]. It is a synthetic, non-toxic and biodegradable thermoplastic polymer with a wide range of applications [7]. Polyethanol used a feather material for thoracic surgery in areas including resins, medicine, the construction industry, and packaging [8]. Antibacterial Applications Due to their biological properties, polymers are of enormous interest [9-10]. Basic ideas about how electromagnetic radiation interacts with both living and inorganic materials [11-12]. Moreover, it is capable of absorbing large amounts of physiological, saline or aqueous solutions, under many environmental factors including ionic strength, temperature, light and pH [13-14]. The inherent qualities of hydrogels, including softness, elasticity, biocompatibility, and responsiveness to stimuli, make them an essential material for a wide range of potential uses in tissue engineering (TE), drug delivery, and wound dressing [15]. A variety of techniques can be used to create hydrogels using monomers containing functional groups such as NH_2, SO_3H, COOH, and OH [16]. Several hydrophilic synthetic and natural polymers have been recently studied as potential possibilities for hydrogel assembly [17]. Hence, hydrogels that possess excellent mechanical strength, non-toxicity, biodegradability, and biocompatibility are highly sought after for many biomedical uses [18-21]. The aim of this work was to create heterogeneous composites by combining the derivatives with polyehanol and evaluate their antibacterial activity; we used bacterial strains to evaluate the antibacterial action.

Experimental

Materials and Methods

The chemicals and their suppliers were utilized exactly as supplied, without additional

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purification. Anthranilic acid, formalin, DMSO, ethyl acetate and pipyridine-1-sulfonyl chloride (Aldrich), ethanol (Riedal-Dehaen 99%), pyridine-3-methylsulphonylchloride (BDH). Melting points in open capillaries were calculated using the Stuart melting point (SMP30, England). ^{1}H-NMR spectra were determined in Iran using a Bruker 300 MHz NMR-spectroscopy. Shimadazu FT-IR spectroscopy using a KBr disc.

Synthesis of 5,5'-methylenedianthranilic acid [22]

The parent compound 4,4'-methylene-bis-(anthranilic acid) was prepared by dissolving anthranilic acid (0.01 mol) in distilled water (10 mL) and 36.5% hydrochloric acid (5 mL) at 50 ^{0}C. The mixture was then treated with 3% aqueous formaldehyde (5 mL) at 20 0C with stirring for 1hour and neutralized with 20% NaOH solution, yellow light precipitate was obtained, washed, deride with hot water and re-crystallized from acetic acid.

Synthesis of 5,5'-methylene bis(2-aminobenzoylchloride)[A][23]:- *according to the Vogel's methods.*

General Procedure I to Synthesis [A1-A4] [23. 24]

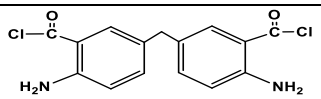
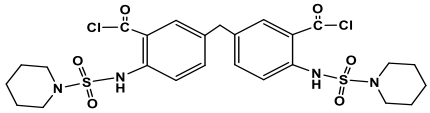
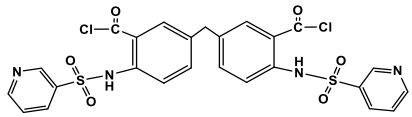
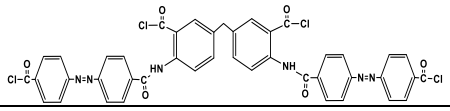
A mixture of (1eq.) of 5,5'-methylene-bis-(2-aminobenzoylchloride) [A], (2eq.) of pipyridine-1-sulphonylchloride and 50 ml of DMSO was added into 100 ml of round bottom flask. Then, the mixture was stirred for 6 hrs., the reaction process was monitored by TLC using chloroform: ethyl acetate [4:6]. The mixture was cooled (by ice bath) to afforded a crude product then, filtered and re-crystallized by mixture of DMSO with ethanol solvent in [1:1].

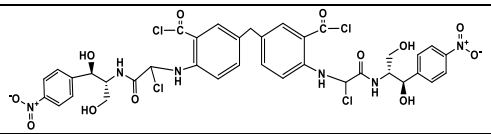
The same way of compound [A1] was repeated with pryidine-3-sulfonyl chloride, 4,4'-(diazene-1,2-diyl) dibenzoyl chloride and chloramphenicol, to prepared compounds [A2, A3 and A4], and the result was shown in Table-1.

General procedure of new Graft-polymers [B1-B4] [23, 25]:

0.2 gm polyethanol was added to a round flask with 50 ml of 70% ethanol with continuous stirring, reaction temperature was kept below 50 °C until the reaction mixture was homogeneous, then 5 drops of conc. H_2SO_4 were gradually added over 1hr. Then, the mixture was left to cool at refrigerator for ½ hr. After that, (0.603 gm, 0.001 mol) of 5,5'-methylene-bis-(2-(phenyl sulfonamido) benzoyl chloride) [A1] was added, the reaction was heated for 3 hrs., another 3 drops of conc. H_2SO_4 were added gradually with continuous stirring and maintaining the temperature below 50°C, the reaction change color, dried, washed and kept in a laboratory dark place to give compound [B1], it was rinsed again to get rid of non-reactive components. The experiment was repeated using the polyethanol with compounds [A2, A3 and A4], to prepared graft-polymer compounds [B2, B3 and B4], illustrate in scheme (3).

Table-1: Some Physic-chemical Properties of Compounds [A-A4].

Comp. No.	Structures compounds	weight	m.p. °C	%	solubility			
					Ethanol	DMSO	DMF	H_2O
[A]		0.90	266	92	+	++	++	+
[A1]		0.68	220	77	++	+++	++	+
[A2]		0.75	245	57	++	+++	++	+
[A3]		0.55	305	80	+	+++	+++	-

[A4]		**0.60**	**256**	**70**	**+**	**+++**	**+++**	**-**

(-):Insoluble less), (+): Weakly (less), (++): Moderately soluble, (+++): Highly soluble (good and above

$$\text{Swelling \% } (\Delta M) = \frac{Mt - M0}{M0} \times 100 \ldots \quad 1$$

Results and Discussion

Synthesized compounds [A-B4] were re-crystallization by mixture of 5:5 DMSO and ethanol solvent, which determined spectral data FT-IR, ^{1}H-NMR and mass spectra [27, 28]. The results of FT-IR showed the appearance of new active groups and the disappearance of active groups, in compound [A1-A4] the free amine group disappeared and the primary amine group appeared, in addition compounds [B1-B4] the acid chloride disappear and ester group appeared to the displacement of the carbonyl group as a result of the introduction of the sulfone-group. FT-IR KBr (cm^{-1}) of compound [A1] as figure (1) showed, 1722sh (C=O), 3215sh (NH), -CH aromatic 3055, CH-aliph at 2922. While FT-IR KBr (cm^{-1}) of compound [A2] showed, (1717sh C=O), (3220sh NH), (-CH aromatic 3090), (CH-aliph at 2982). In addition, FT-IR KBr (cm^{-1}) of compound [A3] showed, (1727sh C=O), (3254sh NH), (-CH aromatic 3100), (CH-aliph at 2922). FT-IR KBr (cm^{-1}) of compound [A4] showed, (1715sh C=O), (3225sh NH), (-CH aromatic 3095), (CH-aliph at 2922).

^{1}HNMR- DMSO-d^{6} of compound [A] appeared (30H), (NH, 2H at δ10.5) ppm, (CH-arom interaction with of CH-bipyridine 26H protons signals at δ7.1-8.6) ppm, and (CH-aliph 2H at δ3.5) ppm. While compound [A2] showed, ^{1}HNMR- DMSO-d^{6} (18H), (NH, 2H at δ11.07) ppm, (CH-arom interaction with CH-pyridine 14H protons signals at δ8.6-9.5) ppm, and (CH-aliph 2H at δ3.6) ppm.

Graft polymer (PVA-g-PIP) compound [B1] was prepared by the reaction of polyethanol with 5,5'-methylenebis(2-(piperidine-1-sulfonamido)benzoyl chloride) in the presence ethanol. A study of FT-IR spectral analysis of compounds [B1], showed stretching bonds C=O at 1729, and NH at 3221, OH bonds at rang 3450sh. As well as PVA-g-PY compound [B2] was prepared by the reaction of polyehanol with 5,5'-methylenebis(2-(pyridine-3-sulfonamido)benzoyl chloride) in the presence ethanol, FT-IR showed stretching bonds C=O at 1720, and NH at 3217, OH bonds at rang 3455sh. While when prepared compound [B3] PVA-g-AZO by the reaction of polyethanol with 5,5'-methylene bis(2-(4-((4-(chlorocarbonyl) phenyl) diazenyl) benzamido) benzoyl chloride) in the presence ethanol, FT-IR showed stretching bonds C=O at 1718, and NH at 3224, OH bonds at rang 3443sh. PVA-g-CHLO compound [B4] was prepared by the reaction of polyethanol with 5,5'-methylene bis(2-((1-chloro-2-(((1R,2R)- 1,3-dihydroxy-1-(4-nitrophenyl) propan-2-yl) amino)-2 -oxoethyl) amino) benzoyl chloride***)***in the presence ethanol, FT-IR showed stretching bonds C=O at 1718, and NH at 3210, OH bonds at rang 3448sh. That is mean the graft polymer may be had homo or random distribution of co-monomeric units in the macromolecule.

In addition the mass spectrum of compound [A1] shown in the Scheme (3), showed an fragment molecular ion at (M^{+}) 617 that refers to the formula showed fragmented ions m/z (618), (616), (420), (304) and (236) respectively, bass peak at m/z (312) in

addition to some of the peaks of the fragments generated from the fusion of the molecule after its ionization probably was considered to be localized at carbon atoms of methylene of the synthesized molecule of symmetrical [A1], [29]. Scheme (3).

XRD-diffraction pattern of compound [B1 and B3], showed the XRD-diffraction studies confirm the successes incorporation of aromatic compounds into the poly-ethanol matrix and showed improved crystallinity following the grafting in poly-ethanol [30, 31]. The figures (2, 3) showed clear peaks of compounds [B1 and B3] at angles ɵ|r (20.4, 553), and (25.09, 765) respectively. This implies that the produced compounds crystalline and semi crystal indicating a regulated in backbone chain.

There are several factors affecting the swelling process, including the relationship between the nature and type of solvent and polymer, the more the relationship is direct, the swelling increases, temperature also affects swelling, as the measurement of swelling differs in the summer from that in the winter and under the same laboratory conditions [26]. The ability to retain water for the longest possible period is very important. Especially in pharmaceutical compounds and in various biomedical fields, the size of the polymer is relative to the material loaded on it [32]. Therefore, we notice that the swelling rate of compound [B1] is 30% greater than that of compound [B2], while we notice that the swelling rate of compound [B4] is 40% greater than that of compound [B3] Because the inter hydrogen bonding between the carbonyl group of ester group and amid group in compound [B1]. and intra hydrogen bonding between the carbonyl group of ester group in others. Figure (4), under the same conditions for both. This is an important vital indicator that the polymer modified with the piperidine moiety compared to the polymer modified with chloramphenicol is less swollen than the latter, which opens the door to loading many pharmaceutical compounds with the same method of action.

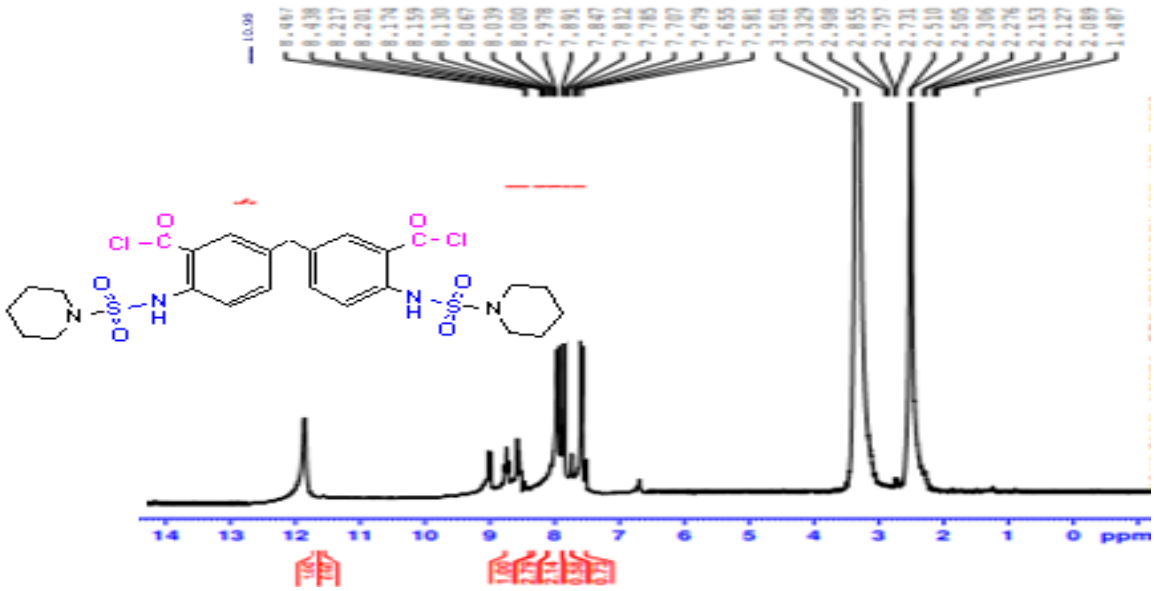
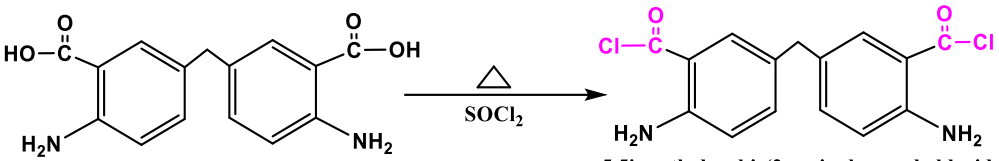
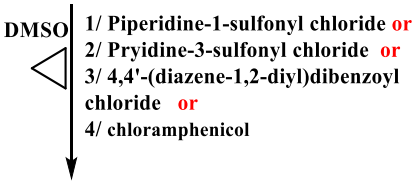
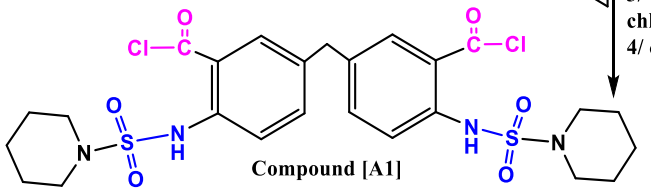


Fig. 1: ^{1}H-NMR spectra of compound [A1].

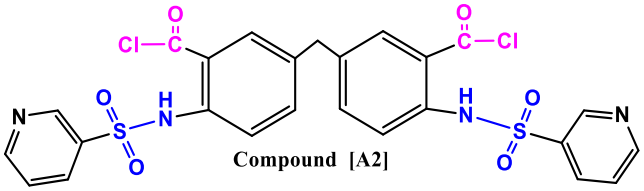


5,5'-methylenebis(2-aminobenzoyl chloride) [A]

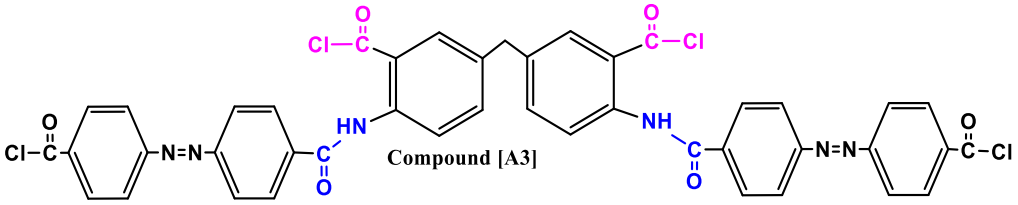




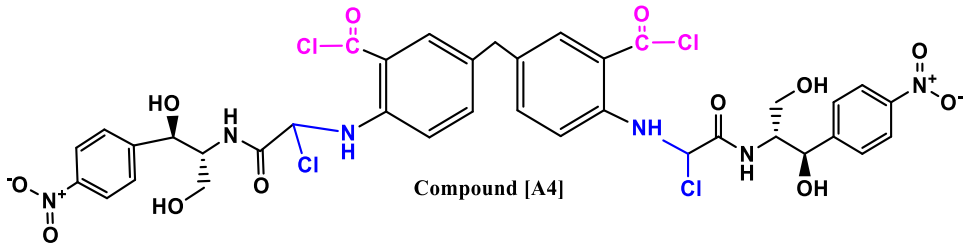
5,5'-methylenebis(2-(piperidine-1-sulfonamido)benzoyl chloride)



5,5'-methylenebis(2-(pyridine-3-sulfonamido)benzoyl chloride)

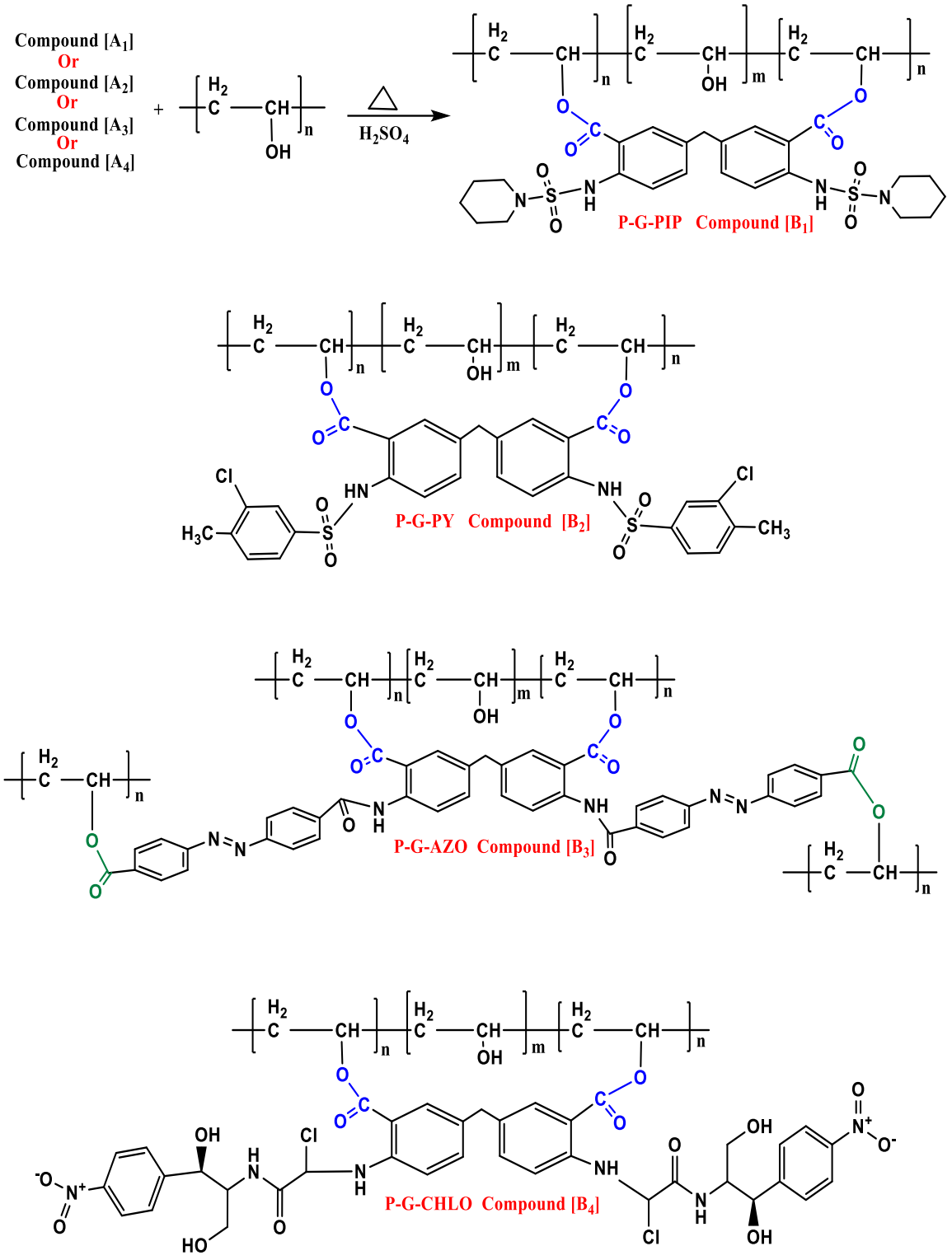


5,5'-methylenebis(2-(4-((4-(chlorocarbonyl)phenyl)diazenyl)benzamido)benzoyl chloride)

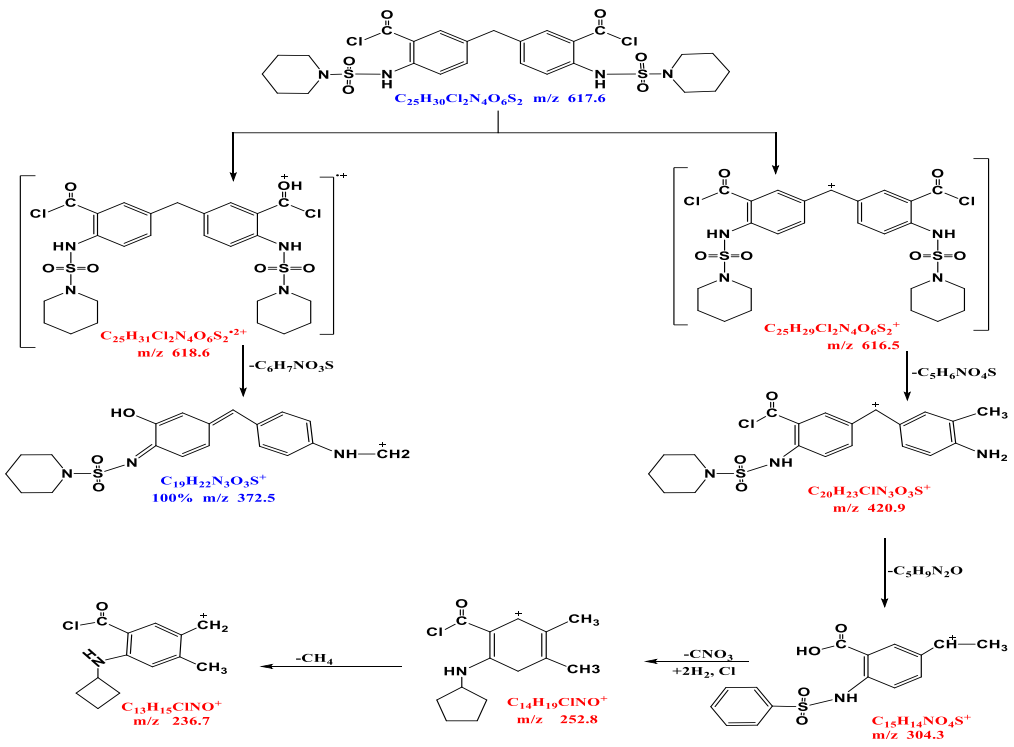


5,5'-methylenebis(2-((1-chloro-2-(((1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl)amino)-2-oxoethyl)amino)benzoyl chloride)

Scheme-1: Synthesis of compounds **[A1, A2, A3 and A4].**



Scheme-2: Synthesis of grafting polymers compounds **[B1, B2, B3 and B4].**



Scheme-3: Suggestion fragment of compound **[A].**

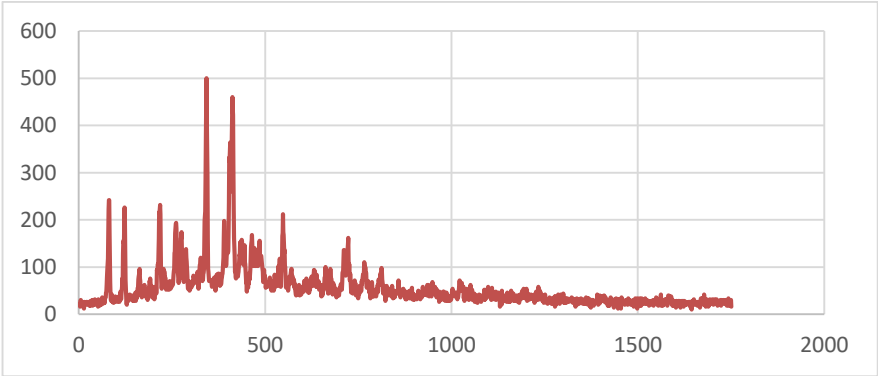


Fig. 2: XRD-diffraction of compound [B1].

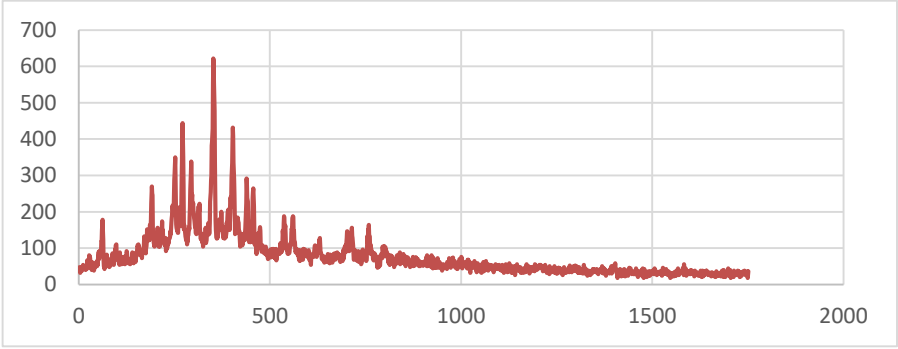


Fig. 3: XRD-diffraction of compound [B3].

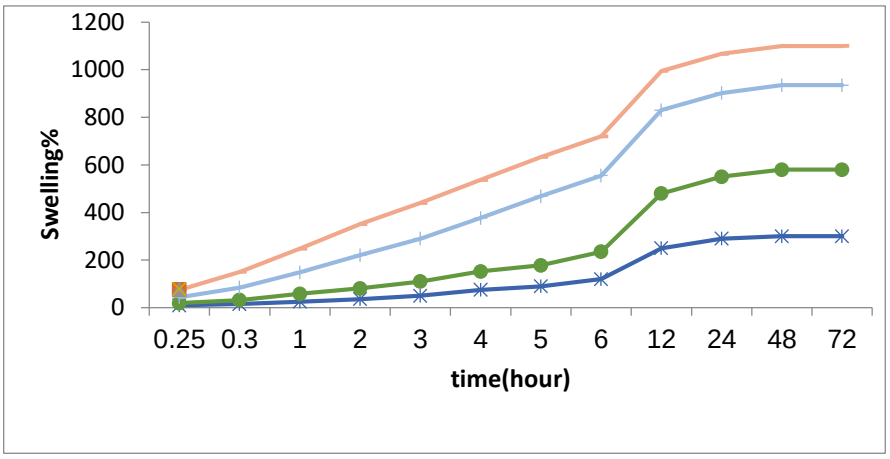


Fig. 4: Swelling of new co-polymers **[B1, B2, B3and B4].**

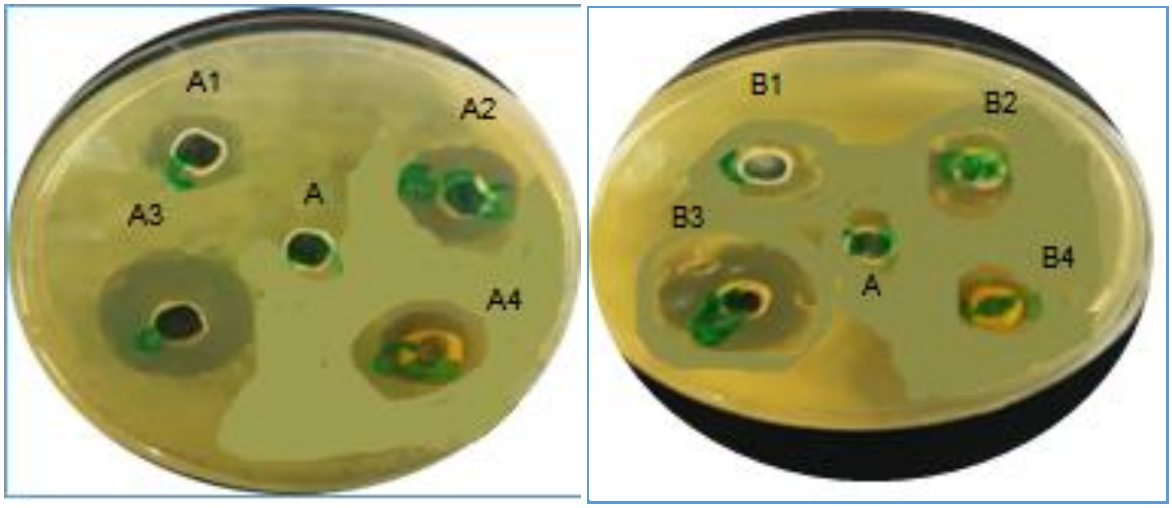


Fig. 5: Antimicrobial Study Inhibition Zone of Compounds **[A-B4]**.

Agar well diffusion method was used to assess the antimicrobial activity of freshly synthesized drugs. In the biology department of the University of Baghdad, all substances were tested for their antibacterial activity against G+Ve *(Staphylococcus aureus)* and G-Ve *(Escherichia coli)* [33-35]. The use of aromatic derivatives loaded on synthetic or natural polymers containing heterocyclic compounds has drawn the attention of researchers because these compounds are active and have a wide range of applications in biological, pharmaceutical, and industrial fields, including antibacterial, antifungal, antimicrobial, antioxidant, and anti-analgesic [36, 37]. Two varieties of G+ve and G-ve bacteria were employed, and the outcomes were reported. Most generated compounds are generally broad and have long-lasting antibacterial activity against bacteria, including *Escherichia coli* G-ve, *Staphylococcus aurous* G+ve, special compounds [A1] showed excellent inhibition against *E-coli*, while compound [B1] showed moderate against *E-coli*, Table-2, Fig. 5.

Table-2: Antimicrobial study inhibition zone of compounds **[A-B4].**

Comp No.	Formula	Mean of Inhibition zone Diameter (mm)	
		Staphylococcus aurous	*Escherichia coli*
A	$C_{43}H_{28}Cl_2N_6O_8$	**28**	**6**
A1	$C_{27}H_{20}Cl_2N_2O_6S_2$	**20**	**4**
A2	$C_{29}H_{24}Cl_2N_2O_6S_2$	**11**	**8**
A3	$C_{43}H_{26}Cl_4N_2O_6$	**26**	**6**
A4	$C_{37}H_{34}Cl_4N_6O_{12}$	**20**	**8**
B1	**PVA-g-PIPY**	**15**	**18**
B2	**PVA-g-PYR**	**14**	**24**
B3	**PVA-g-AZO**	**10**	**6**
B4	**PVA-g-CHLO**	**8**	**3**
DMSO	C_2H_6OS	-	-

Conclusion

By reacting bis-methylene chloride with various hetero-aromatic compounds [A-A4] were created compounds [B1-B4] using traditional methods and reacted with polyethanol using the cold-warm esterification method. The new poly functional polymers, which were created by adding initiators to create linear random graft polymers, have a number of advantageous characteristics. They demonstrated good antimicrobial activity, good swelling and solubility in common solvents, good running thermal stability by enhancing some of the mechanical properties of the polymer by incorporating polyvinyl alcohol with various compounds that mimic the conditions of acidic lipophilic and hydrophilic foods.

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