

Synthesis, characterization of poly(ester-amide) biodegradable and evaluation of their antimicrobial activity

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ABSTRACT

In this study, Biodegradable poly(ester amide) (PEA) were synthesized by the melt polycondensation of adipic acid (AA) and 1,2-bis(2-oxazoliny-2) butane (BBO). The solubility of the polymer was checked in different polar and non-polar solvents, their chemical structures were confirmed by FT-IR, ¹H-NMR and ¹³C-NMR spectroscopic techniques, and the biodegradability of this polymer prepared by chain linking was demonstrated using different microorganisms. The PEA was screened for antimicrobial activity against three different pathogenic bacteria Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa*; *Escherichia coli*) by using the cut plug method and viable cell counting methods. The results obtained showed that PEA has antimicrobial activity against gram negative bacteria (*E.coli*) as well as gram positive bacteria (*S.aureus*), and showed intermediate inhibition against gram negative bacteria (*P.aeruginosa*).

Keywords: poly(ester amide), characterization, biodegradability, antimicrobial activity.

1. INTRODUCTION

Biocidal polymer is a polymer that has the ability to kill microorganisms, by acting as a source of sterilizing ions or molecules [1]. Generally, the use of conventional antimicrobial agents is associated with the problems of residual toxicity of these agents which can cause more serious problems to the environment [2], as result there is an increase in the demand of new devices for the water treatment. One of the possibilities to avoid this phenomenon is the development of materials having an antibacterial activity such as polymers. Indeed the use of polymers having an antibacterial activity offers an advantage of minimizing the problems of contamination of the environment especially that of the water pollution by the chemical agents with low molecular weight. Polymers are used as biocidal agents in recent times. By incorporating biologically active organic moieties into the polymer backbone, the activities can be introduced in terms of their biological activity; these polymers are more effective than their monomers. Such polymers are known for their biocidal activity against some bacterial, fungal and viral strains [3]. Reuben Jonathan reported the antibacterial activity of certain poly(ester-amides) synthesized from 4, 4'-oxybis (benzoic acid) [4]. Poly(ester-amide)s is new class of polymers that combine the good degradability of polyesters with the high thermal stability, high

modules and high tensile strength of polyamides [5]. Polymers that undergo a controlled biological degradation by microorganisms have become of remarkable interest during the last years [6]. Biological degradation is generally considered as a phenomenon of biological transformation of organic compounds by living organisms particularly microbes. It has been considered as a natural process in the microbial world as carbon and energy source for their growth and takes a key role in the recycling of materials in the natural ecosystem [7].

This paper deals with the synthesis, characterization and investigation of biodegradability and bactericidal activity of poly(ester-amide) by direct polycondensation of bizoxazoline (BBO) with adipic acid. This PEA was characterized with a variety of experimental techniques including qualitative solubility tests, spectral studies and biodegradability tests. Agar well diffusion method was employed to study the antibacterial activity of PEA against different types of microorganisms including Gram-positive bacteria (*S. aureus*), Gram-negative bacteria (*P. aeruginosa*; *E. coli*) and Macrodilution method for the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).

2. EXPERIMENTAL SECTION

2.1. Materials and methods.

Chem-lab samples of Adiponitrile (AN), Ethanolamine (EA), and zinc di-acetate hydrated were used as received. Adipic acid (AA) was supplied from Aldrich chemical and used as received. These materials were of analytical grade, and were used for poly(ester-amide) (PEAd) polyesterification without further purification. Methanol, ethanol, heptane were also purchased from

Aldrich Chemical Co. Chloroform, toluene, Tetra hydro furane (THF) and Dimethyl sulfoxide (DMSO) that were used as solvents were of analytical grade from Fluka. Solvents were used as supplied by commercial sources without any further purification.

Solubility of the poly(ester-amides) was determined in various solvents qualitatively, for PEA sample in excess of solvent at different temperature. The IR spectrum of the PEA was

recorded on a Perkin-Elmer FTIR spectrometer. Spectra were collected in the region of 500–3500 cm^{-1} . The $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ spectra of PEA were recorded at room temperature on Bruker spectrometer operating at a frequency of 300 MHz in DMSO-d_6 solution, with TMS as the internal standard.

2.2. Chemistry.

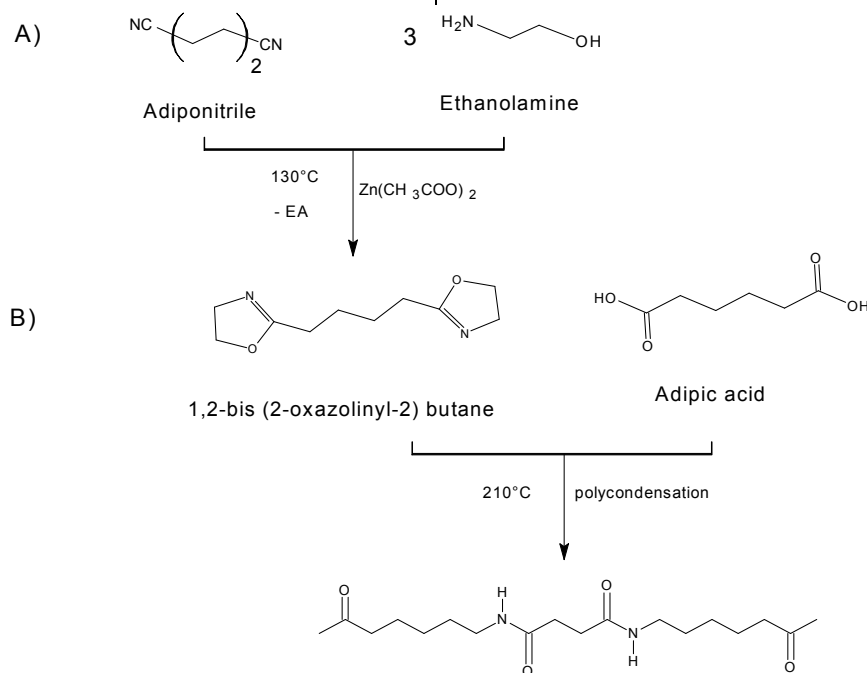
2.2.1. Preparation of 1, 2-bis (2-oxazoliny-2) butane « BBO ».

A mixture of 1 mole of adiponitrile, 3 moles of ethanolamine and 0.025 mole zinc di-acetate hydrate was refluxed at 130°C . Ammonia was evolved and swept from the system with nitrogen. After 4 hours, the reaction mass was then vacuum

distilled at 180°C . At 0.3 mm Hg to recover unreacted materials. The pot residue product solidified to an amorphous wax whose (Scheme 1.A) [8].

2.2.2. Synthesis of poly(ester-amide) (PEA).

The PEA was prepared by a coupling reaction of adipic acid and the bizoxazolines (Scheme 1.B). We drove this reaction of polyaddition an equimolar ratio between adipic acid and coupler of chains BBO under inert atmosphere (N_2) at 210°C during 120 min. The poly(ester-amide) thus obtained was in form of semisolid mass of color dark brown. Yield was 90%.



Scheme 1. A) Synthesis of bisoxazoline with polycondensation method, B) Coupling reaction between AA and BBO.

2.3. Pharmacology.

2.3.1. Microbial degradation of poly(ester-amides).

All tested strains were from patients of Sidi Bel Abbes University hospital, and were authenticated by Microbiological Laboratory of the Biological Institute of Sidi Bel Abbes University [9, 10]. Bacterial strains were: the Gram-negative bacteria *Escherichia coli*, and *Pseudomonas aeruginosa*, the Gram-positive bacteria *Staphylococcus aureus*, *Bacillus Subtilis*, and the filamentous fungi *Aspergillus niger*. The fungus was isolated from moldy jam.

2.3.1.1. Minimal Medium Preparation.

Fungal Culture: The culture was grown in minimal medium (ASTM Nutrient Salts Medium) containing (g/l): KH_2PO_4 0.7; K_2HPO_4 0.7; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.7; NH_4NO_3 1.0; NaCl 0.005; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.002; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.002; MnSO_4 , H_2O 0.001. The pH of the medium was adjusted to 6.5 ± 0.5 .

Bacterial Culture: Cultures were grown in Minimal medium (OECD) containing (g/l): $(\text{NH}_4)_2\text{SO}_4$ 2.0; K_2HPO_4 14.0; KH_2PO_4 6.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2. The pH of the medium was adjusted to 6.4 prior to sterilization.

The same components were used for the preparation of the liquid media only the medium was supplemented with 14 g/l agar

to generate solid medium. The solid and liquid medium were inoculated an oven for 20 min (1200°C , 2 bars). The mixture was autoclaved in a 250 ml conical flask at 121°C for 20 min. The stock cultures of the microorganisms were stored on solid culture medium (25 ml), in Petri dishes, maintained at 4°C in the refrigerator.

2.3.1.2. Testing in solid media.

The bacterial strains (*basilus subtilis*) and the fungi (*Aspergillus niger*) were screened by culturing on Petri dishes containing 25 ml of solid culture medium with the addition of poly(ester amide) and without (control culture). After the medium sterilization in the autoclave, the agar was cooled to $40-45^\circ\text{C}$ and the microorganisms from recent cultures were transferred to the surfaces of the agar plates and the poly(ester amide)s were deposited on the surface. The Plates were incubated for 48 h at 30°C [11].

2.3.1.3. Testing in liquid media.

Two small slices of solid medium (1.2 cm x 1.2 cm) were born bearing the microorganisms were transferred to test-tube containing 20 ml of liquid mineral medium, previously sterilized in autoclave for 20 minutes at 121°C . Next, the poly(ester amides)

were added to the medium, and was incubated for 48 h at 30°C [12].

2.3.2. Evaluation of the antimicrobial activity.

2.3.2.1. Quality screening.

Quality screening of microbial strains sensitivity to PEA was performed with an adapted agar diffusion method [13]: Petri plates were prepared by pouring 10 ml of Muller Hinton Agar for bacteria and allowed to solidify. These agar plates were inoculated with 0.1 ml of standardized bacterial suspension (2×10^6 cells/ml) and uniformly spread. A 6 mm well was cut and filled with 10% DMSO of synthetic compounds. A well filled with 10% DMSO served as control. After 48 h of incubation at 37°C. The bactericidal effect of product (bacterial growth inhibition) was measured by the appearance of a zone of inhibition (clear zone) around the spot. The diameter of the inhibition zone observed around the well was measured for each bacterium.

2.3.2.2. Quantitative testing.

Quantitative testing of the antimicrobial activity of PEA was performed by Macro-dilution method in liquid medium for the determination of the MIC, MBC.

Minimum inhibitory concentrations (MIC): One of the earliest antimicrobial susceptibility testing methods was the macrobroth or tube-dilution method [14]. This procedure involved preparing serial dilutions were made for selected polymers in order to prepare concentrations of 10^{-3} , 5.10^{-3} , 10^{-2} , 5.10^{-2} , and 10^{-1} mg/ml zero concentration was considered as a negative control. Antibacterial activity is usually tested by making aqueous solution of the compounds. However, the polymers used in the present study were insoluble in water, and hence their solutions were prepared in DMSO. DMSO was inactive towards the selected micro-organisms as demonstrated from blank experiment carried out with DMSO alone and tested thereafter. Therefore, to study

antibacterial activity of the complexes, their solutions were prepared in DMSO.

A previously prepared pure spore suspension of each tested microorganism (0.5 ml of about $1-5 \times 10^5$ CFU/ml) was mixed with 9.5 ml of each concentration in sterile test tubes, incubated at 32°C for 24 h, the tubes were examined for visible bacterial growth as evidenced by turbidity and of the surviving cells (% Optical density). The lowest concentration of antibacterial that prevented growth represented the minimal inhibitory concentration (MIC).

The Minimum Bactericidal Concentration (MBC): The MBC for each strain was taken from the concentration of the lowest dosed test tube showing visually no growth after 24 h. 10 µl from each visually ungrown test tube were sub cultured on a nutrient agar medium [15]. MBC endpoints were read as the lowest dilution of drug with no growth (>99.9% killing) after overnight incubation at 35°C.

Measurements of antibacterial activity in water: The measurement of microbial kill requires the ability to measure the number of surviving microorganisms with time after exposure to the antibacterial agent. This test has the same requirement of Time-kill method [16].

For this experiment used MIC selected to prepare diluted polymers. A previously prepared suspension of each tested microorganism, were grown overnight in nutrient broth at 37°C. 1 ml of each suspension (of about 10^8 cells/ml) was mixed with 9 mL of diluted PEA in sterile test tubes, incubated at 37°C, then optical density of growth was measured by spectrophotometer (UV-VIS-1202 SHIMADZU) at 600 nm every 12 h for each incubated mixture, we used both optical density (OD, 600 nm) and colony forming unit (CFU) data. The results were represented graphically and optical density at time=0 was considered as a negative control.

3. RESULTS SECTION

The purpose of the current work is to synthesize poly(ester-amide) and to evaluate its bactericidal efficacy. Poly(ester-amides) are a category of polymeric materials which contains both ester and amide linkages and are synthesized by the polymerization of a diacid (AA) with that of a bisoxazoline (BBO) as chain extender in the mole ratio of 1:1.

Table 1. Solubility behavior of PEA.

Solvent	PEA	
	RT	BP
Water	-	-
Methanol	-	-
Ethanol	-	-
THF(Tetra hydro furane)	-	-
Toluene	-	-
Heptane	-	-
Chloroform	-	∓
DMSO	+	+

Room temperature (RT) boiling point (BP) soluble (+) insoluble (-) swillable (∓).

The PEA is soluble in one polar aprotic solvent (DMSO) but insoluble with the other solvents used in this test. At elevated temperatures the PEA1 turn swillable in Chloroform (table.1).

3.1. Infrared spectral studies.

Poly(ester-amide) was prepared by The coupling reaction between BBO and AA as illustrated in Scheme 1. BBO react with carboxylic acids through the ring opening between positions 1 and 5 of the oxazoline, producing compounds possessing both amide and ester bonds. This has been confirmed by both FT-IR spectral analyses, as shown in Figure 1. The FTIR spectra shows the absorption bands at 1728.45 cm^{-1} and 1420.23 cm^{-1} that were attributed to the linear ester and carboxyl terminal groups respectively. The amide bands were noted respectively at 1545.73 cm^{-1} . The signal at 1630.14 cm^{-1} was attributed to the CO of the amide.

3.2. ¹H-NMR spectra.

Confirmation of the coupling reaction between BBO and adipic acid is provided by ¹H NMR rectoscope (Fig.2). The existence of a grouping methylene linked to an amide function of reacted bisoxazoline moieties give resonances at 3.35 ppm; they

also note protons close to a function ester appear in 4.66ppm. The spectrum shows appearance signals of (4H, CH₂CH₂CONH)

apparenteront to 2.261 ppm and 2.05. Protons (4H, CH₂CH₂COO) will be more reinforced and resound in 1.43ppm.

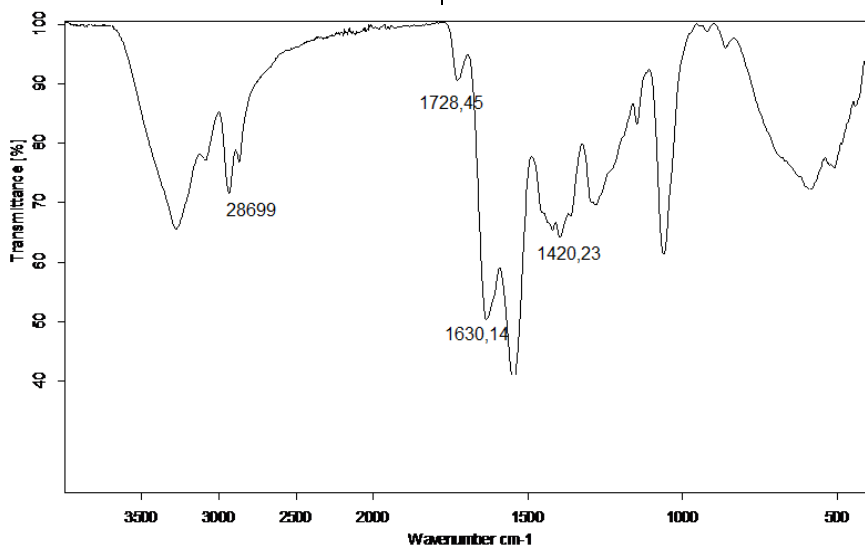


Figure 1. FTIR spectra of PEA.

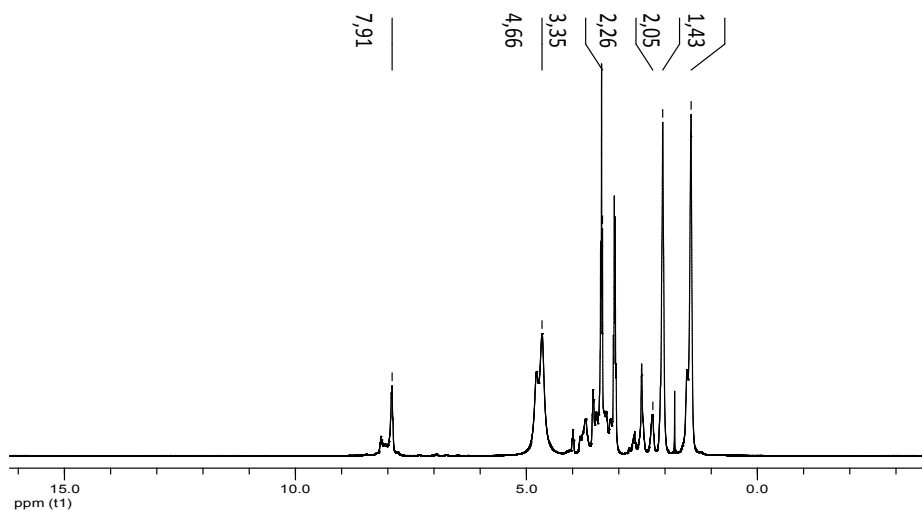


Figure 2. ¹H-NMR spectrum of PEA.

3.3. ¹³C-NMR spectra.

The structure of poly(ester-amide) recovered after reaction of polyaddition of adipic acid with BBO, was identified through comparison of the ¹³C-NMR spectra of Adipic acid, BBO and PEA. According to ¹³C-NMR analysis of the poly(ester-amide),

the spectrum (Figure 3) showed the partial disappearing of the peak C=O corresponding to the carboxyl (174,29 ppm) of adipic acid in the chain extending reaction and new peak C=O of the ester group (172,54 ppm) was formed. The new peak was detected at 159.77 ppm correspond the oxamide carbonyl of opened EBO.

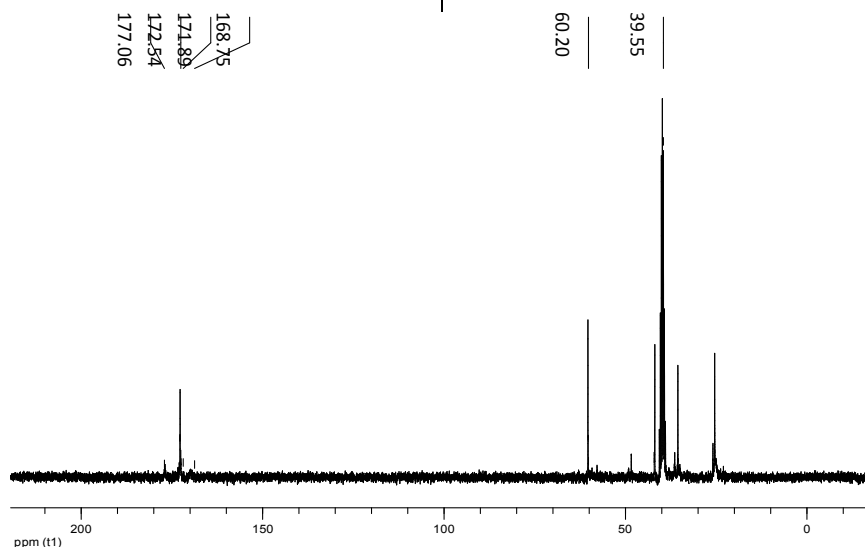


Figure 3. ¹³C-NMR spectrum of PEA.

Another peaks the peaks could be seen in the poly(ester-amide) spectrum, corresponding the rest of the reactive, the peak was detected at 168.75 which confirms that not all of the BBO monomer reacted during the chain extending reaction. The presence of peaks corresponding the carboxyles groups unreacted watches that reaction is not complete.

3.4. Results of Biodegradation.

The study of the biodegradation of PEA was conducted with bacterial (*Bacillus subtilis*) and fungus (*Aspergillus niger*) in sterilize basal mineral salt medium. The visual growth of the bacterial and the fungus is seen from the photograph in Figure 4.

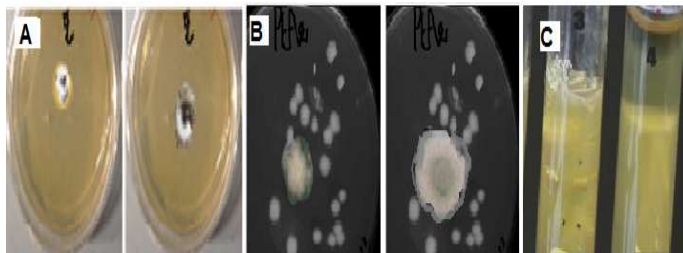


Figure 4. (A) PEA disintegrated at the end of 3 days with *A. Niger*. (B) Evolution the mediums of biodegradation of the PEA after 3 days with *B. Subtilis*. (C) Liquid media swirled to show growth of bacterial.

After 2 days, we noted a clear zone of hydrolysis on solid media inoculated with *Bacillus subtilis* strain. These bacterial showed good potential for biocatalytic degradation of polymer. But with *Aspergillus niger*, we observed less disintegrated of polymer. We noted a zone hydrolysis not very important, therefore they conclude there was a bacterial activity but it learns of time. Biodegradation of PEA was the most important in the liquid medium showed by *Bacillus subtilis* produces flocculent (flaky, clumped) growth in liquid media but the degradation by the fungus was negligible. Then, biodegradation of polymer is dependent the type of microorganisms, in this study the bacterial showed more capability to degrade PEA compared with the fungi, and also dependent the chemical and physical characteristics of the polymer. The chemical structure (responsible for functional group stability, reactivity, hydrophylicity and swelling behaviour) is the most important factor affecting the biodegradability of polymeric materials [17].

The test cultures used in the present study are organisms widely found in the environment. The mechanism of degradation is not known exactly. The PEA is viewed by microorganisms as an energy sources [18]. Microorganisms can damage the structure and function of synthetic polymers. According to Flemming [19].

During degradation, exo-enzymes from microorganisms break down complex polymers yielding smaller molecules of short chains, e.g., oligomers, dimers, and monomers, that are smaller enough to pass the semi-permeable outer membranes of the microbes, and then to be utilized as carbon and energy sources [18, 20].

3.5. Antimicrobial activities.

Polymeric materials are known for their antimicrobial activities. In the present work, the bacterial activity of PEA was assayed against *Escherichia coli*, *Staphylococcus aureus*, and *P. aeruginosa* by used Disc diffusion method (Kirby-Bauer) is recommended as qualitative method and Tube dilution method is used to determine (MIC) and (MBC) (quantitative method).

3.5.1. The agar disc diffusion method.

After 24 h, the diameters for the zone of inhibitions at different concentration against the test bacteria are given in Table 2.

The results reported in Table 2 showed that the PEA had substantial antimicrobial activity against the three bacteria tested with different degrees. In fact, the diameters of the growth inhibition zone ranged from 16 mm (*E. coli*) to 12 mm (*S. aureus*). The lowest diameter of inhibition growth zone was recorded for *P. aeruginosa* (05 mm).

Table 2. Inhibition effects of the poly(ester-amide) on the growth of the three bacteria tested after 24 h and after 72 h.

Microorganisms	Gram	PEA2		
		24h		48h
		Inhibition zone (mm)	Results	Inhibition zone (mm)
<i>E. coli</i>	-	16	S	17
<i>P. aeruginosa</i>	-	05	R	7.5
<i>S. aureus</i>	+	12	I	13

Resists (R)<8 mm; Intermediary resistance (I)9-14 mm; Sensitive (S)>15mm.

The *E. coli* strain was the more sensitive to this polymer, a second gram negative bacterium showed resistance.

On the other hand, *S. aureus* (gram positive bacterium) has sensitive to PEA

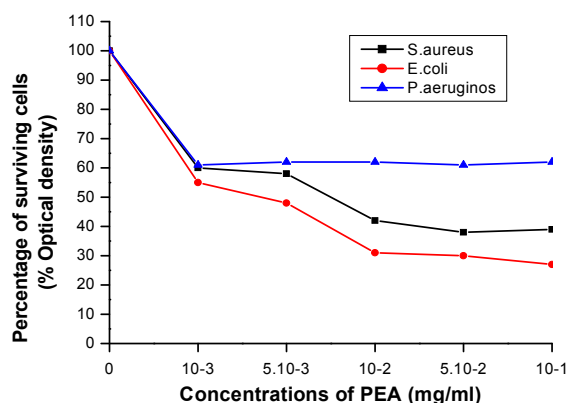


Figure 5. Growth inhibition of different concentrations of PEA against different tested microorganisms.

After 48h, all polymers showed an increase in the inhibitory action against all the tested microorganisms. Also, it was observed that the highest activity was recorded against *E. coli* with varying magnitudes (17mm); the lowest was recorded against *P. aeruginosa* (7.5mm), and the zone of inhibition of *S. aureus* (13mm). Then the PEA was less efficient against bacterium *P. aeruginosa*. This Differences in may be related to differential susceptibility of bacterial cell wall, which is the functional barrier

to minor differences present in the outer membrane in the cell wall composition [21].

3.5.2. Minimum inhibitory concentration determination for the polymer.

The MIC was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms [22]. The growth-inhibiting effect was quantitatively determined by percentage of the surviving cells (% Optical density) as shown in (Figure 5). The MIC values for this polymer were determined by using the broth dilution method. We considered zero concentration as a negative control. The polymer concentrations

ranged from 10^{-3} - 10^{-1} mg/ml which was obtained by serial dilutions. Each solution in the series was mixed with 10^5 cells/ml of each tested microorganism.

MIC was recorded at 10^{-2} mg/ml against *E. coli* and increased to 5.10^{-2} mg/ml against *S. aureus*, but *P. aeruginosa* is less sensitive than the other two, since a higher MIC value was obtained with this bacteria.

Like previous tests, the application of the dilution broth method confirms by its results shown in Table 3 the important antibacterial activity of the PEA on these three microbial strains, as it seems that *E. coli* is more sensitive than the other two.

Table 3. Minimum Inhibitory Concentration of PEA using the dilution broth method against the three bacterial strains.

Bacterial strain	Control	DMSO	Concentration of PEA (mg/ml)				
			10^{-3}	5.10^{-3}	10^{-2}	5.10^{-2}	10^{-1}
<i>S. aureus</i>	+++	+++	+++	++	+++	+	+
<i>E. coli</i>	+++	+++	++	++	+	+	+
<i>P. aeruginosa</i>	+++	+++	++	++	+++	+++	+++

++: Comparable growth with that Control +; slow growth.

3.5.3. The Minimum Bactericidal Concentration (MBC).

The MBC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no development of turbidity and without visible growth [23]. MBC results of the poly(ester-amide)s is given in Table 4, The results show that MBC of PEA for used *S. aureus*, *E. coli*, was 10^{-1} , 5.10^{-2} mg/ml respectively. But *P. aeruginosa* seemed the more resistant; the strain was able to survive at higher concentrations of this polymer.

Table 4. Minimum Bacterial Concentration (MBC) of PEA using the dilution broth method against the three bacterial strains.

Bacterial strain	concentration of PEA (mg/ml)				
	10^{-3}	5.10^{-3}	10^{-2}	5.10^{-2}	10^{-1}
<i>S. aureus</i>	+	+	+	+	-
<i>E. coli</i>	+	+	+	-	-
<i>P. aeruginosa</i>	+	+	+	+	+

No growth (-) growth(+)

3.5.4. Antibacterial activity in water.

Bacteria suspension with an optical density (OD) of 0.07 at 600 nm (measured by a UV-vis spectrophotometer), which corresponded to the approximate cell density of McFarland Standard solution (10^8 CFU/ml).

The results of antimicrobial assay at the following contact times are presented as the OD and CFU of bacteria solutions after an inoculum density of $\sim 10^8$ CFU ml $^{-1}$ being treated with MIC levels of and incubating in different times, plus negative (bacteria/cell suspension), and positive (bacteria suspension treated with PEA controls for comparison as shown in Figure 6. For both bacteria strains, the PEA performed intermediate inhibition strength against microorganisms, where the differences

were still significant with around 20 and 43% reduction for *E. coli* and *S. aureus*, respectively, no significant difference can be observed with *P. aeruginosa* compared to the negative control.

Within 48 h, colony forming unit numbers of both strains with the PEA were reduced below the minimum detectable level ($\sim 10^5$ CFU ml $^{-1}$), it can be seen that populations of $\sim 1 \times 10^8$ CFU ml $^{-1}$ *P. aeruginosa* not decreased after 48h incubation with this PEA. However, no bacterial re-growth was observed with both strains in this time.

These results indicated that MIC levels of tow PEA were capable of producing a readily detectable effect against high cell densities with two types of bacterium *S. Aureus* and *E.coli*, but was ineffective to kill *P. aeruginosa*.

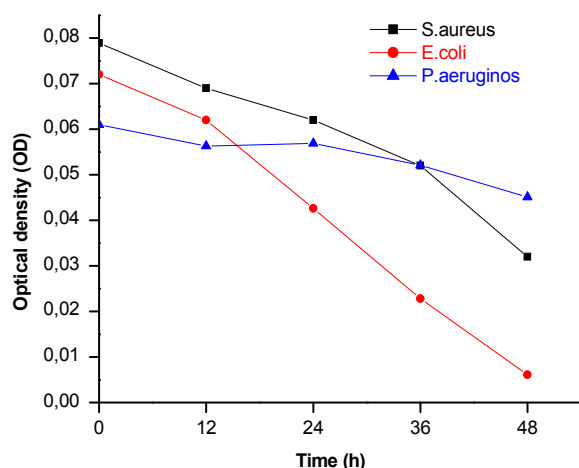


Figure 6. Growth inhibition of PEA against different tested microorganisms after different time with a fixed concentration.

Table 5. The effect of MIC levels of PEA on a population of $\sim 1 \times 10^8$ CFU ml⁻¹ different Bacterial strains.

Bacterial strain	Concentration (MIC) of PEA mg/ml	Control	Colony forming unit (CFU) studies in different time (h)			
			12	24	36	48
S. aureus	5.10 ⁻²	1.1×10 ⁸	0.85×10 ⁸	8.8×10 ⁷	7.1×10 ⁷	4.2×10 ⁷
E. coli	10 ⁻²	10 ⁸	0.84×10 ⁸	5.7×10 ⁷	2.8×10 ⁷	8.5×10 ⁷
P. aeruginosa	10 ⁻¹	0.8×10 ⁸	0.71×10 ⁸	7.1×10 ⁷	7×10 ⁷	5.7×10 ⁷

4. CONCLUSIONS

Poly(ester-amide)s PEAs have been developed as promising biodegradable material since they combine a degradable character caused by the existence of hydrolysable ester groups (-COO-) in their backbone with relatively good thermal and mechanical properties afforded by the strong intermolecular hydrogen bonding interactions established between their amid group (-NHCO-) [5].

In this study, focused on the synthesis of novel poly(ester-amide)s biodegradable by polymerization BBO and adipic acid. The ester and amide functional groups present in the poly(ester-amide) chain were identified by FT-IR spectra. The structural units present in the poly(ester-amide) chain were identified by ¹H and ¹³C NMR spectra.

We further explored the biodegradation behaviors of poly(ester amide) with different strain, biodegradation of PEA showed that polymer are fully biodegradable with the bacterial and the fungi but There was a difference between the size of the colony formed on the surface of the plates, and the growth of strain in the tube relative to the control culture. Polymers that

undergo a controlled biological degradation by micro-organisms have become of remarkable interest during the last years. Microorganisms play a significant role in biological decomposition of materials, including synthetic polymers in natural environments [24].

In our study; PEA were used as novel antimicrobial agents; were screened against *E. coli*, *P. aeruginosa* and *S. aureus* bacteria. The results of present antimicrobial assay revealed that the PEA showed good inhibitory activity against two the tested pathogens (*E. coli* and *S. aureus*) and a weak activity with *P. Aeruginosa*, but showed poor bactericidal effect against the strains tested.

This polymer has a good solvent resistance, the prepared biocidal polymers are water-insoluble; therefore, they can be used safely in sterilizing drinking water and many other applications, such as disinfecting water supplies, swimming pools, hot-tubs, industrial water systems, and other applications where a sanitized water supply is required [25].

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