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Research Article

BIOCHEMISTRY

Synthesis and characterization of modified Poly(Ethylene-Co-Vinyl Alcohol) and its application as a biocidal agent

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KEY WORDS ABSTRACT

P(EVOH),

Biocidal, Chloroacetylation, Antimicrobial

polymers

Co-polymeric materials of Poly (Ethylene-Co-Vinyl Alcohol) with different ratios, P (EVOH), were chemically modified through a series of reactions including chloroacetylation followed by the amination reaction. The acetyl, amine and sulfide groups were estimated through the Fourier Transform Infra-Red Spectra (FT-IR) and the Elemental analysis. The antimicrobial activity of the modified copolymers was tested against various species by the cut plug procedure. It was concluded that the modified copolymers exhibited antimicrobial activity towards both Gram positive and Gram negative bacteria. The highly observable result was estimated towards the *protous* species, at a ratio of 27% Ethylene and 73% Vinyl Alcohol since the inhibition zone reached 41 mm. The antimicrobial activity increased by increasing the percentage of ethyl groups in the copolymeric material. So, the synthesized polymers were used as biocidal agents for water treatment.

Introduction

The microbiological contamination of water causes various negative effects on the human health, because of the spread of infections, and the quality control difficulties of the microbial

corrosion and fouling, caused by the biofilm layer [1]. The conventional methods for water treatment concludes different mechanical and physical process [2, 3]. Furthermore, the chemical treatments have been applied, such as the application of different agents (disinfectants, chemical biocides) to inactivate microorganisms [4, 5]. Chlorination is the most applied treatment that involves some difficulties, as the evaluation of toxic byproducts [6]. The infection by pathogenic microorganisms has a great potential in various applications, particularly in the medical fields, health care, and hygienic applications, textiles, water treatment, dental surgery and food industries [7], [5]. Antimicrobial agents are defined as materials that are able to kill pathogenic microorganisms [8]. The application of antimicrobial copolymers decreases the environmental impact related to the conventional antimicrobial agents by the reduction of their residual toxicity, and hence optimization of their through controlling efficacy their lifetime. Moreover. the antimicrobial polymers are nonvolatile chemically stable agents that cannot invade through the skin. In this area, infections related to the biomaterials, signify a certain task to the medical implants [9-11]. Efficient copolymers is referred to both the functional groups and to their polymeric nature that their features be influenced by the extraordinarily size of the polymeric molecules [12-14]. Antimicrobial mediators are applied for various applications, for instance water disinfection, medications, sterilization, and the inhibition of the growth[13],[15-21]. Ethylene microbial vinyl alcohol copolymer (EVOH) is a semi-crystalline copolymer of ethylene and vinyl alcohol monomeric subunits. P(EVOH) is a well-known flexible thermoplastic copolymer for packaging, funnel tanks, and different medical techniques [22]. The synthesis of EVOH has been adjusted depending on their applications, for example, films, nanoparticles, and nanofibers [23, 24]. Several reasons for the selection of EVOH in this research. First, the polymeric material is highly hydrophilic because of the vinyl alcohol subunits. Second, it is a waterinsoluble referred to the ethylene subunits. Third, the good biocompatibility of EVOH; and finally, the other hydroxyl functionality exhibits chances for

chemical modification [2, 25, 26]. Actually, several literatures have demonstrated the application of EVOH as a biomaterial [27]. Juliana and Maria [28] reported that P(EVOH) different ethylene with percentage and poly(methyl methacrylate) (PMMA) were synthesized by the mechanical grinding in the melted phase. Roberto et al [29] demonstrated the single- screw extrusion procedure to obtain composite films of P(EVOH). The recent research aimed to investigate the synthesis of a novel antimicrobial copolymers of P(EVOH) for water treatment.

2. Experimental

2.1. Materials and Methods

Poly(ethylene-co-vinyl alcohol), purity 99% was purchased from Sigma Aldrich, Germany. Chloroacetyl chloride and Glacial acetic acid were purchased from Elgoumhouria Co. (Cairo, Egypt). Pyridine was purchased from El-Nasr Pharmaceutical Chemicals (Egypt). Ethylene diamine (EDA) and Propylene diamine (PDA) were purchased from Acros (New Jersey, USA).

2.2. Test microorganisms and media

The tested microorganisms were Escherichia coli, Streptococcus aureus, Klepsiella sp and Proteus sp in addition to some fungi such as Asperigillus niger and pathogenic Candida spp. (C. krusei, C. albican, C. tropicalis and C. glabrata). The tested microorganism was obtained from the Bacteriology and Mycology unit, Botany Department, Faculty of Science, Tanta University. 2.3. Chloroactylation of poly (ethylene co vinyl alcohol)

Pyridine (55 mL) was added to a suspension of EVOH (27%, 32%, 38% and 44%) (10 g) in 100 mL DMSO and the mixture was cooled to 0°C. Chloroacetylchloride (55 mL) was added dropwise and stirred at 0 °C for 3 h and room temperature for further 4 days. The insoluble part was removed by filtration and the filtrate was collected and concentrated on the rotary evaporator. The product was precipitated by adding distilled water, filtered off washed with water and was dried under vacuum at 40°C for two days.



Scheme 1: Chloroactylation of poly(ethylene co vinyl alcohol)

2.4. Amination of chloroacetylated poly(ethylene-co-vinyl alcohol)2.4.1. Amination with Ethylene diamine

A suspension of copolymer (1) (3 g) is added to 30 mL of ethylene diamine. The reaction mixture was stirred at 90°C for 7 h, then remain the reaction mixture to complete for 24 h. The product was filtered off, washed with distilled water then with ethanol to remove the excess of ethylene diamine. The product was dried at 40 °C under vacuum for two days.



Copolymer (2)

Scheme 2: Reaction of copolymer (1) with Ethylene diamine

2.4.2. Amination with propylene diamine

The titled compound was prepared as described earlier for Ethylene diamine using the following quantities: 3 g of copolymer (1) and 30 mL of propylene diamine.



Scheme 3: Reaction of copolymer (1) with Propylene diamine

2.5. The reaction with carbon disulphide

2.5.1. The reaction of Copolymer (2) with carbon disulphide

Copolymer (2) was dissolved in ethanol then Sodium hydroxide (0.01 mole) was added. The mixture was heated till miscibility and then 20 mL of carbon disulphide was added dropwise. The full treatment of amine group with carbon disulphide was confirmed by using lead acetate, the reaction medium was neutralized by adding diluted HCl till PH=7. The product was filtered off, dried under vacuum at 40 °C for two days



Scheme 4: Reaction of copolymer (2) with carbon disulfide

2.5.2. The reaction of Copolymer (3) with carbon disulphide

The titled compound was prepared as described earlier for ethylene diamine using the following quantities: aminated copolymer (3), Sodium hydroxide (0.01 mole) and 20 mL of carbon disulfide.



Scheme 5: Reaction of copolymer (3) with carbon disulfide

2.6. Characterization of polymeric materials

The acetyl, amine and sulfide groups were estimated through Fourier Transform Infra-Red Spectra (FT-IR), Perkin–Elmer, England) and Elemental analysis (Heraeus, elemental analyzer model 1106 Carlo ErbaStrmentazione).

3. Results and discussion

3.1. Characterization of the synthesized polymeric materials 3.1.1. FT-IR Spectra

FT-IR spectra were characterized by the presence of a broad peak around 3420 cm⁻¹ due to O-H stretching of R₂CHOH which was confirmed by a peak around 1119 cm⁻¹ corresponding to C-O stretching. The presences of peaks around 1631 cm⁻¹ are due to the formation of ester bond. The appearance of a peak around 1755 cm⁻¹ give an indication to the formation of carbonyl group in copolymer (1) [30]. The chloroacetylation step was confirmed by the appearance of peak around 750 cm⁻¹ for (C-Cl) and Peaks appeared around 1459 cm⁻¹ were due to CH₂ stretching. The FT-IR spectra of Copolymer 2,3) confirm the reaction with ethylene diamine and propylene diamine which evaluate the amine stretching group at 2930 cm⁻¹ as a winged peak [31]. The carbon disulphidereaction was insured by the thiocarbonyl group stretching around 1055 cm⁻¹.



Figure (1): FT-IR of the synthesized compounds

3.1.2 Elemental microanalyses

Table (1) showed the different percent of Nitrogen ratio in the different copolymer (2). From these results, it is confirmed that with increasing the percentage of ethyl alcohol in the copolymer leads to increase the nitrogen ratio in the copolymers (2,3) after amination. Also, it leads to increase the nitrogen and sulfur ratio in the copolymers (4,5) (Table 2). The amination process was confirmed with the elemental microanalysis of nitrogen in the copolymers. It is clear that the increase in the copolymeric material from 27% to 32% causes drop in the nitrogen content from 10.3% to 9.49% while the lowest nitrogen ratio (6.82%) was recorded at 44% of ethyl alcohol in the polymeric material. It is confirmed that with increasing the percentage of ethyl alcohol in the copolymer leads to increase the nitrogen and sulfur ratio in the copolymer.

Table (1): Elemental analysis of Copolymer (2)

Copolymer (2)	C%		H%		N %	
	Cal	Exp	Cal	Exp	Cal	Exp
27% Ethylene	55.8	48.78	9.3	7.11	11.88	10.3
32% Ethylene	56.0	51.44	9.4	7.71	11.07	9.49
38% Ethylene	57.2	53.37	9.5	8.1	10.09	7.9
44% Ethylene	58.4	49.42	9.7	9.12	9.1	6.82

Table (2): Elemental analysis of Copolymer (4)

Copolymer (4)	С	%	Н	.%	Ν	%	S	%
	Cal	Exp	Cal	Exp	Cal	Exp	Cal	Exp
27% Ethylene	40.0	35.7	5.5	4.1	7.5	6.0	17.3	15.9
32% Ethylene	41.1	37.4	5.6	4.1	7.0	5.4	16.1	13.1
38% Ethylene	42.2	34.3	5.7	5.1	6.4	4.1	14.6	11.8
44% Ethylene	43.3	38.4	5.9	5.7	5.8	3.6	13.2	8.9

 Table (3): Elemental analysis of Copolymer (3)

Copolymer (3)	С%		H%		N %	
	Cal	Exp	Cal	Exp	Cal	Exp
27% Ethylene	58.1	52.42	9.67	7.23	10.99	10.27
32% Ethylene	59.3	49.43	9.72	7.75	10.23	8.18
38% Ethylene	60.5	57.77	9.8	8.25	9.33	7.86
44% Ethylene	61.8	55.54	9.9	9.58	8.43	5.9

 Table (4): Elemental analysis of Copolymer (5)

Copolymer (5)	С	%	H	%	Ν	%	S	%
	Cal	Exp	Cal	Exp	Cal	Exp	Cal	Exp
27% Ethylene	42.2	38.1	5.9	5.4	7.1	6.8	16.4	14.3
32% Ethylene	43.4	41.5	6.1	5.7	6.7	6.5	15.3	14.1
38% Ethylene	44.5	40.2	6.2	5.7	6.1	4.5	13.9	11.2
44% Ethylene	45.6	39.0	6.3	6.0	5.5	3.6	12.6	8.5

3.2. Antifungal activities

It was found that the modified polymers exhibited antifungal activity against tested fungi as Aspergillusniger, Candida albican, C. krusi, C. glabrata and C. tropicales. Table (5) showed the difference in the diameter of inhibition zones of different concentrations of copolymer using ethylene diamine in the amination. Candida albican has the largest diameter of inhibition zone 14 mm with 27% ethylene. C. tropicals had the smallest diameter of inhibition zone 5 mm with 44 % ethylene which confirmed that the antimicrobial activity increased with increasing the concentration of copolymer (the conc. of vinyl alcohol). This behavior has been reported from previous researches [32]. From table (6), the difference in the diameter of inhibition zones from 27% to 44% copolymer using propylene diamine. A.niger has the largest diameter of inhibition zone 28 mm with 27% using propylene diamine. C.tropicals had the smallest diameter of inhibition zone 3 mm with 32% using propylene diamine which confirmed that the antimicrobial activity increased with increasing the concentration of copolymer (the conc. of vinyl alcohol).

Table (5): Diameters of inhibition zones (mm) ofmodified Poly(Ethylene-co-vinyl alcohol) with variouschemical structure using ethylene diamine againstdifferent test Fungi

Tested Fungi	27% Ethylene	32% Ethylene	38% Ethylene	44% Ethylene
Candida albican	14	12	11	1
C.Krusi	0	0	0	0
C.glabrata	12	9	8	5
C.tropicals	6	11	9	5
A.niger	13	11	9	6

Table (6): Diameters of inhibition zones (mm) of modified poly (Ethylene-co-vinyl alcohol) with various chemical structure using propylene diamine against different test Fungi

Tested micro organism	27% Ethylene	32% Ethylene	38% Ethylene	44% Ethylene
Candida albican	25	22	0	0
C.Krusi	0	0	0	0
C.glabrata	11	5	0	0
C.tropicals	9	3	0	0
A.niger	28	11	8	7



Figure 2: Inhibition on linear growth of modified copolymers with ethylene diamine against the selected pathogenic fungi



Figure (3): Inhibition on linear growth of modified copolymers with propylene diamine against the selected pathogenic fungi

3.3. Antibacterial activities

It was found that the modified polymers exhibited antibacterial activity against tested Gram negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella sp* and *Proteus sp*) and Gram positive bacteria (*Staphylococcus aureus*).**Table (7)** showed inhibition zones of 27 % to 44% copolymer using ethylene diamine in which *Protous* has the largest diameter of inhibition zone 41 mm with 27% ethylene. *Protous* has the smallest diameter of inhibition zone was 5 mm with 44% ethylene which confirm that the antibacterial activity increased with increasing the concentration of copolymer (the conc. of vinyl alcohol).

The inhibition zones of 27 % to 44% copolymer using propylene diamine showed that *Protous* has the largest diameter of inhibition zone 32 mm with 27 % using propylene diamine, *E.coli* has the smallest diameter of inhibition zone 2 mm with 44 % using propylene diamine which confirmed that the antibacterial activity increased with increasing the concentration of copolymer (the conc. of vinyl alcohol) **Table (8)**.

Table (7): Diameters of inhibition zones (mm) of modified poly (Ethylene-co-vinyl alcohol) with various chemical structure with ethylene diamine against different test bacteria

Tested bacteria	27% Ethylene	32% Ethylene	38% Ethylene	44% Ethylene
Klepisa	30	28	21	17
Escherichia coli	21	18	14	12
Protous	41	28	12	5
Staphylococcus aureus	18	13	11	8

Table (8): Diameters of inhibition zones (mm) of modified poly (Ethylene-co-vinyl alcohol) with various chemical structure with propylene diamine against different test bacteria

Tested micro organism	27% Ethylene	32% Ethylene	38% Ethylene	44% Ethylene
E.coli	23	22	6	2
S.aureus	12	9	6	3
Klepisla	29	16	2	0
Protous	32	21	12	10



Figure (4): Inhibition on linear growth of modified copolymers with ethylene diamine against the selected pathogenic bacteria



Figure 5: Inhibition on linear growth of modified copolymers with propylene diamine against the selected pathogenic bacteria

4. Conclusions

The modification of the Poly(Ethylene-Co-Vinyl Alcohol) was carried out by reacting them with chloroacetyl chloride followed by amination with ethylene diamine and Propylene diamine.The newly synthesized compounds exhibit remarkable antimicrobial activities against series of fungi as (C. krusei, C. albican, C. tropicalis and C. glabrata) and Aspergillus nigerin addition to bacteria as (E. coli, Streptococcus aureus, Protoussp and *Klepislasp*). Copolymers possessed a highly observable antimicrobial activity against all the tested fungi and bacteria for all the prepared concentrations, as it ranged in case against fungi from inhibition zone diameters of 3 mm for the concentration of 32% using propylene diamine against C.tropicals to inhibition zone diameters of 28 mm for the concentration of 27% using propylene diamine against A.niger and from 1 mm for concentration 44% using ethylene diamine againstC. albican to 14 mm with concentration 27 % using ethylene diamine against C.albican. On the other hand, it was ranged in case of bacteria from inhibition zone diameters of 5 mm for the concentration of 44% using ethylene diamine against protous sp to inhibition zone diameters of 41 mm for the concentration of 27% using ethylene diamine against protous sp and from 2 mm for concentration 44% using propylene diamine against E.coli to 32 mm with concentration 27 % using propylene diamine against protous sp

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