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Abstract

The aim of this work is synthesis of Poly (Vinyl-4-AminoBenzoate) (PVAB) from reaction of Poly Vinyl Alkohol PVA with 4-aminobenzoyl chloride in alkaline media. We also prepare the metal complexes of poly (vinyl-4-aminobenzoate) and antimicrobial properties were evaluated by dilute method against five pathogenic bacteria (Escherichia coli, Shigella dysentery, Klebsiella pneumonae, Staphylococcus aureus, Staphylococcus Albus) and two fungal (Aspergillus Niger, Yeast). All polymer metal complexes showed different activities against the various microbial isolates. The polymer metal complexes showed higher activity than the free polymer.

Keywords: PVA, polyester, antibacterial, antifungal, activity.

Introduction

The control of the growth of microbes, such as bacteria, fungi, yeast and algae, in nature is one of the fundamental concepts for the survival of higher species. Plants, animals, even microbes themselves have developed a great variety of mechanisms that keep microbes at bay [1]. In human society these control mechanisms often do not work efficiently, which makes microbial infections the number one killer in the world. The treatment of microbial infections becomes more and more difficult, because the number of resistant microbial strains as well as that of antibiotic-immune patients grows a lot faster than the number of useable antibiotics [2, 3].

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Antimicrobial polymers represent a class of biocides that has become increasingly important as an alternative to existing biocides and in some cases even to antibiotics. The working mechanism of the large number of structurally different polymers is often not fully understood. However, some of them are known for their low potential of building up resistant microbial strains [4].

Polyesters have attracted significant interest as drug carriers due to their biocompatibility and biodegradability [5, 6]. They were started to be used as synthetic polymer material in the mid-1950, they have a great chemical and biological durability. Their elasticity, resistance to weather, abrasive strength, low susceptibility to sorption, distortion strength, no toxicity, resistance to heat treatment in sterilization, moderate capillarity, gave the polyesters their present wide usability in various fields of surgery [7].

Experimental

Synthesis of Poly (Vinyl-4-Amino Benzoate) (PVAB)

4-Aminobenzoic acid (0.5g, 3mmol) was dissolved in SOCl₂ (2ml, 26.9mmol) and refluxed for 5hrs. The excess of SOCl₂ was evaporated under vacuum to obtained quantitative yield of 4-Aminobenzoyl chloride. The 4-Aminobenzoyl chloride was co-evaporated with diethyl ether to remove traces of SOCl₂. The resulting 4-Aminobenzoyl chloride was dissolved in Di Methyl Sulf-Oxide DMSO (5ml) and to this solution of Poly Vinyl Alcohol (PVA) (0.5g), in DMSO (10ml), KOH (0.5g, 8mmol) was added. The reaction mixture was stirred for 48 hrs at room temperature. After completion of the reaction, the mixture was poured into ice-water and chloroform was added to extract the materials were not reacted. The residue was evaporated to yield the ester (PVAB) (0.89g), as a pale brown solid. FTIR (KBr, cm⁻¹): 3444 (NH₂), 3000 (C-H_{at}.), 2926 (-CH₂-), 2856 (-CH-), 1688 (C=O_{est.}), 1604 (C=C_{at.}), 1419 (-CH-asym.), 1315 (-CH-sym.), 1261-1111 (C-O_{est.}), 900-600 δ(C-H_{at.}) (O.O.P.) [8].

Preparation of the poly (vinyl-4-aminobenzoate) metal complexes

The Silver nitrate (AgNO₃) and Mercury chloride (HgCl₂) were obtained from Fluka. Nickel chloride (NiCl₂.6H₂O), Manganese sulfate (MnSO₄.H₂O), Cobalt chloride (CoCl₂.H₂O) and Copper chloride (CuCl₂.2H₂O) were obtained from Aldrich. Sabouraud Agar, Blood Agar Base, MacConky Agar and Nutrient Broth were obtained from Oxoid LTD.

General procedure for the preparation of metal complex

The general procedure for preparation of metal complex by preparing 5% from polymer solution and mixed with equal ratios of metal solution (Mn, Co, Ni, Cu, Hg, Ag) (10 mmol), mixture was stirred for 1 hr.

Evaluation testing of antimicrobial activity:

Antimicrobial susceptibility test measures the ability of an antimicrobial

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agent to inhibit or kill bacterial growth in vitro. This ability may be estimated by either the dilution method or the diffusion method. In this work we followed the broth dilution method. Certain bacteria and fungi isolates were chosen, *Escherichia-Coli*, *Shigella dysentery* and *Klebsiella Peneumoniae* were representing gm-ve isolates, *Staphylococcus aureus* and *Staphylococcus albeus* were representing gm+ve isolates, two fungal (*Aspergillus niger*, *Yeast*). Those Isolates were taken from about 50 patients at CPHL (Central Public Health Laboratory in Baghdad).

The broth dilution method: Serial twofold dilutions of an antimicrobial agent are incorporated into broth containing tubes that are then inoculated with a standard number of organisms usually (10⁵-10⁶) Colony-Forming Units (CFU) per milliliter. After the culture has been incubated at 37C⁰ for 18 hr. The lowest concentration that prevents growth after overnight incubation is known as the Minimum Inhibitory Concentration (MIC) of the agent. The MIC is defined as the lowest concentration of antimicrobial agent at which there is no visible growth [9, 10].

Results & Discussion

PVAB was prepared from treatment of polyvinyl alcohol with 4-aminobenzoyl chloride in presence of potassium hydroxide and DMSO as solvent with stirring for 48 hours (scheme 1). The FTIR spectrum for compound (PVAB) exhibited the band at (3344) cm⁻¹ due to (NH₂) and (1688) cm⁻¹ due to (C=O_{est.}) and disappearance the band at (3415) cm⁻¹ due to (OH) groups for PVA spectrum.

Scheme (1): Preparation of the poly (vinyl-4-aminobenzoate)

Antimicrobial studies

Antimicrobial activity of the synthesized compound and its corresponding metal complexes was determined against three Gram-negative bacterial strains (*Escherichia coli*, *Shigella dysentery* and *Klebsiella Pneumoniae*), two Grampositive bacterial strains (*Staphylococcus aureus* and *Staphylococcus albus*) and two fungal (*Aspergillus Niger* and *Yeast*) Tables (1-7) and (8-14) respectively.

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Table (1): Minimum inhibitory concentration (µg/ml) of (PVAB) against bacteria iso
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Isolates	St. Gr					С	oncentra	ation μ	g/ml				
isolates	Gram Stain	550	600	650	700	750	800	850	900	950	1000	1050	1100
Escherichia Coli	-ve	+	+	+	+	+	-	-	-	-	-	-	-
Shigella dysentery	-ve	+	+	+	+	+	+	+	-	-	-	-	-
Klebsiella Pneumoniae	-ve	+	+	+	+	+	+	+	-	-	-	-	-
Staphylococcus aureus	+ve	+	+	+	+	+	+	+	+	-	-	-	-
Staphylococcus albus	+ve	+	+	+	+	+	+	+	+	+	+	-	-

Table (2): Minimum inhibitory concentration (μg/ml) of (PVAB-Hg) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml													
isolates	am ain	10	20	30	40	50	100	150	200	250	300	350	400		
Escherichia Coli	-ve	+	-	-	-	-	-	-	-	-	-	-	-		
Shigella dysentery	-ve	+	-	-	-	-	-	-	-	-	-	-	-		
Klebsiella Pneumoniae	-ve	+	-	-	-	-	-	-	-	-	-	-	-		
Staphylococcus aureus	+ve	+	+	-	-	-	-	-	-	-	-	-	-		
Staphylococcus albus	+ve	+	+	-	-	-	-	-	-	-	-	-	-		

Table (3): Minimum inhibitory concentration (µg/ml) of (PVAB-Ag) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml													
Isolates	am ain	50	100	150	200	250	300	350	400	450	500	550	600		
Escherichia Coli	-ve	+	-	-	-	-	-	-	-	-	-	-	-		
Shigella dysentery	-ve	+	-	-	-	-	-	-	-	-	1	-	-		
Klebsiella Pneumoniae	-ve	+	+	-	-	-	-	-	-	-	-	-	-		
Staphylococcus aureus	+ve	+	+	-	-	-	-	-	-	-	-	-	-		
Staphylococcus albus	+ve	+	+	+	-	-	-	-	-	-	-	-	-		

Table (4): Minimum inhibitory concentration (μg/ml) of (PVAB-Cu) against bacteria isolated.

Isolates	Gram Stain				<u> </u>	Con	centrat	ion με	/ml				
Isolates	am ain	150	200	250	300	350	400	450	500	550	600	650	700
Escherichia Coli	-ve	+	1	-	-	-	-	-	-	-	-	-	-
Shigella dysentery	-ve	+	-	-	-	-	-	-	-	-	-	-	-
Klebsiella Pneumoniae	-ve	+	+	-	-	-	-	-	1	-	-	-	-
Staphylococcus aureus	+ve	+	+	-	-	-	-	-	_	-	-	-	-
Staphylococcus albus	+ve	+	+	-	-	-	-	-	-	-	-	-	-

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able (5): Minimum inhibitor	4 4 .	/ / I\ C	ADTIAD (C.)	
 able (5): Wiinimiim inhihifar	a concentration (ing/milant	TPVAR-COLSC	gainst hacteria isolated
 ADIC (5), ITAHIHHHUH HIHHDICUL	· concentration :	LUZIIII) VI	AT ALTO COLUM	<u> z</u> amsi bacici ia isolaica.

Isolates	m m					Con	centra	ition µ	ιg/ml				
isolates	n 12	450	500	550	600	650	700	750	800	850	900	950	1000
Escherichia Coli	-ve	+	+	+	+	+	-	-	-	ı	-	-	-
Shigella dysentery	-ve	+	+	+	+	+	+	-	-	-	-	-	-
Klebsiella Pneumoniae	-ve	+	+	+	+	+	+	-	-	-	-	-	-
Staphylococcus aureus	+ve	+	+	+	+	+	+	+	ı	ı	ı	ı	-
Staphylococcus albus	+ve	+	+	+	+	+	+	+	-	-	-	-	-

Table (6): Minimum inhibitory concentration (µg/ml) of (PVAB-Ni) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml												
Isolates	am	450	500	550	600	650	700	750	800	850	900	950	1000	
Escherichia Coli	-ve	+	+	+	+	+	-	-	-	1	1	-	-	
Shigella dysentery	-ve	+	+	+	+	+	+	-	-	-	-	-	-	
Klebsiella Pneumoniae	-ve	+	+	+	+	+	+	+	-	1	1	-	1	
Staphylococcus aureus	+ve	+	+	+	+	+	+	+	+		- 1	-	1	
Staphylococcus albus	+ve	+	+	+	+	+	+	+	+			-	-	

Table (7): Minimum inhibitory concentration (μg/ml) of (PVAB-Mn) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml												
isolates	am ain	450	500	550	600	650	700	750	800	850	900	950	1000	
Escherichia Coli	-ve	+	+	+	+	+	+	+	-	-	-	-	-	
Shigella dysentery	-ve	+	+	+	+	+	+	+	+	-	-	-	-	
Klebsiella Pneumoniae	-ve	+	+	+	+	+	+	+	+	-	-	-	-	
Staphylococcus aureus	+ve	+	+	+	+	+	+	+	+	-	-	-	-	
Staphylococcus albus	+ve	+	+	+	+	+	+	+	+	+	+	-	-	

Table (8): Minimum inhibitory concentration (µg/ml) of (PVAB) against fungi isolated.

Igalatas	Concentration µg/ml Isolates													
Isolates	1000	000 1050 1100 1150 1200 1250 1300 1350 1400 1450 1500												
Aspergillus Niger	+	+	+	+	+	+	+	+	-	-	-			
Yeast	+	+	+	+	+	+	+	_	-	-	-			

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Table (9): Minimu	Table (9): Minimum inhibitory concentration (μg/ml) of (PVAB-Hg) against fungi isolated.													
Isolates					Conc	entratio	n μg/m	1						
isolates	750	50 800 850 900 950 1000 1050 1100 1150 1200 1250												
Aspergillus niger	+	+	-	-	-	-	-	-	-	-	-			
Yeast	+	+	+	-	-	-	-	-	-	-	_			

Table (10): Minimum inhibitory concentration (μg/ml) of (PVAB-Ag) against fungi isolated.

T 1.					Conc	entratio	n μg/m	1			
Isolates	750	800	850	900	950	1000	1050	1100	1150	1200	1250
Aspergillus niger	+	+	+	-	-	-	-	-	-	-	-
Yeast	+	+	+	+	+	-	-	-	-	-	-

Table (11): Minimum inhibitory concentration (µg/ml) of (PVAB-Cu) against fungi isolated.

Isolates	Concentration μg/ml										
	750	800	850	900	950	1000	1050	1100	1150	1200	1250
Aspergillus niger	+	+	+	ı	-	-	-	-	-	-	-
Yeast	+	+	+	+	+	+	-	-	-	-	-

Table (12): Minimum inhibitory concentration (µg/ml) of (PVAB-Co) against fungi isolated.

Isolates	Concentration µg/ml											
	850	900	950	1000	1050	1100	1150	1200	1250	1300	1350	
_	ergillus iger	+	+	+	+	-	-	-	-	-	-	-
Ye	east	+	+	+	+	+	+	-	-	-	-	-

Table (13): Minimum inhibitory concentration (μg/ml) of (PVAB-Ni) against fungi isolated.

Isolates	Concentration µg/ml										
	850	900	950	1000	1050	1100	1150	1200	1250	1300	1350
Aspergillus niger	+	+	+	+	-	-	-	-	-	1	ı
Yeast	+	+	+	+	+	-	-	-	-	-	

Table (14): Minimum Inhibitory Concentration (μg/ml) of (PVAB-Mn) against fungi isolated.

Isolates	Concentration µg/ml										
	1000	1050	1100	1150	1200	1250	1300	1350	1400	1450	1500
Aspergillus niger	+	+	+	+	+	+	+	ı	-	-	-
Yeast	+	+	+	+	+	+	ı	ı	-	ı	-

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The synthesized PVAB and all polymer complexes exhibited a vary degree of inhibitory effects on the growth of different bacterial and fungi isolates. PVAB has amine groups that have a large biological effectiveness and that are present in a many number of medications. Antimicrobial agents may affect cells in a variety of ways, many of which are poorly understood [11]. Most of the commonly used antibacterial chemotherapeutic agents act by one of the following basic mechanisms: competitive antagonism of some metabolite, inhibition of bacterial cell wall synthesis, action on cell membranes, inhibition of protein synthesis, or inhibition of nucleic acid synthesis [12].

The Compounds (PVAB-Hg) and (PVAB-Ag) were, however, found to be active against all the bacteria and fungi. To the contrary, the compound (PVAB), (PVAB-Cu), (PVAB-Ni) and (PVAB-Mn) was found to be low active against the all bacteria and fungi.

The complexes Polymer metal showed higher activity than the free metal, these results substantiate our own finding and the findings of some other workers that biologically inactive compounds become active and less biologically active compounds become more active upon coordination [13,14], this may be due to, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to which liposolubility is an important factor that controls antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity in turn enhances the penetration of the complexes into lipid membranes and blocking of metal binding sites on the enzymes of the microorganisms. The metal complex may also be a vehicle for activation of the ligand as the cytotoxic agent [15].

In the other side the presence of some compound in the microbial agent which have some groups like (-SH,-NH₂, -COOH,-OH) that attracts the metal elements (Mn, Co, Ni, Cu, Hg, Ag) to form specific chelate complexes and thus it will increase the lipophilicity of the complexes which in turn will facilitate concentration in the bacterial cell, where the eventual action is to impair their ability to synthesis protein on the ribosomes. Irrespective of the fact that Ni and Co were introduced in higher molar amounts and Cu was added in the smallest concentration, according to potency as antimicrobial agents the PVAB chelates can be arranged in the following order:

Hg chelate > Ag chelate > Cu chelate > Co chelate > Ni chelate > Mn chelate
Other factors such as solubility, conductivity and dipole moment as
influenced by the presence of metal ions may also be amongst the possible

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reasons causing enhancement of the bactericidal activity of the metal complexes as compared to the uncomplexed ester compounds [16,17].

The fungi were found to be completely resistant to the polymeric preparation in this research irrespective of the fact that it was successful as antibacterial agents. It has been found that prepared metal polymeric complex compounds give better result when used as antifungal drugs, but in undesirable level to be considered as antifungal.

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تحضير ودراسة الفعالية الحيوية لمعقدات (Mn, Co, Ni, Cu, Hg) مع بولي (فاينيل –4 بنزوات الامين)

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الخلاصة

يتضمن هذا البحث تحضير بولي (فاينيل -4- بنزوات الامين) من تفاعل بولي فاينيل الكحول مع (4- امينو كلوريد البنزويل) في وسط قاعدي، وتحضير معقدات لهذا المشتق مع المعادن واختبار الفعالية التثبيطية بأتباع طريقة التخفيف القياسية ضد خمسة انواع من البكتريا (Escherichia coli, الفعالية التثبيطية بأتباع طريقة التخفيف القياسية ضد خمسة انواع من البكتريا (Staphylococcus aureus, Shigella dysentery, ونوعين من الفطريات (Staphylococcus Albus). اظهرت جميع معقدات البوليمر فعاليات مختلفة ضد مختلف انواع الميكروبات وفعالية اعلى من البوليمر نفسه. الكلمات المفتاحية: بولي فاينيل الكحول، بولي استر، مضادات البكتريا، مضادات الفطريات.