Synthesis and Antimicrobial Activities of (Mn, Co, Ni, Cu, Hg and Ag) Complexes with Poly (Vinyl-٤-Aminobenzoate)

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Abstract

The aim of this work is synthesis of Poly (Vinyl-٤-AminoBenzoate) (PVAB) from reaction of PVA with ٤-aminobenzoyl chloride in alkaline media. We also prepare the metal complexes of poly (vinyl-٤-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 [١]. 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 [٢, ٣].

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 [٤].

Polyesters have attracted significant interest as drug carriers due to their biocompatibility and biodegradability [٥, ٦]. They were started to be used as synthetic polymer material in the mid-١٩٥٠, 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 [٧].

Experimental

Synthesis of Poly (Vinyl-٤-Amino Benzoate) (PVAB)

٤-Aminobenzoic acid (٠,٥g, ٣mmol) was dissolved in $SOCl_2$ (٢ml, ٢٦,٩mmol) and refluxed for ٥hrs. The excess of $SOCl_2$ was evaporated under vacuum to obtained quantitative yield of ٤-Aminobenzoyl chloride. The ٤-Aminobenzoyl chloride was co-evaporated with diethyl ether to remove traces of $SOCl_2$. The resulting ٤-Aminobenzoyl chloride was dissolved in DMSO (٥ml) and to this solution of poly Vinyl Alcohol (PVA) (٠,٥g), in DMSO (١٠ml), KOH (٠,٥g, ٨mmol) was added. The reaction mixture was stirred for ٤٨ 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) (٠,٨٩g), as a pale brown solid. FTIR (KBr, cm^{-1}): ٣٤٤٤ (NH_2), ٣٠٠٠ ($C\text{-}H_{ar.}$), ٢٩٢٦ ($-CH_2-$), ٢٨٥٦ (-CH-), ١٦٨٨ ($C=O_{est.}$), ١٦٠٤ ($C=C_{ar.}$), ١٤١٩ ($-CH\text{-}_{asym.}$), ١٣١٥ ($-CH\text{-}_{sym.}$), ١٢٦١-١١١١ ($C\text{-}O_{est.}$), ٩٠٠-٦٠٠ δ($C\text{-}H_{ar.}$) (O.O.P.) [٨].

Preparation of the poly (vinyl-٤-aminobenzoate) metal complexes

The Silver nitrate ($AgNO_3$) and Mercury chloride ($HgCl_2$) were obtained from Fluka. Nickel chloride ($NiCl_2.6H_2O$), Manganese sulfate ($MnSO_4.H_2O$), Cobalt chloride ($CoCl_2.H_2O$) and Copper chloride ($CuCl_2.2H_2O$) 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 ٥٪ from polymer solution and mixed with equal ratios of metal solution (Mn, Co, Ni, Cu, Hg, Ag) (١٠ mmol), mixture was stirred for ١ hr.

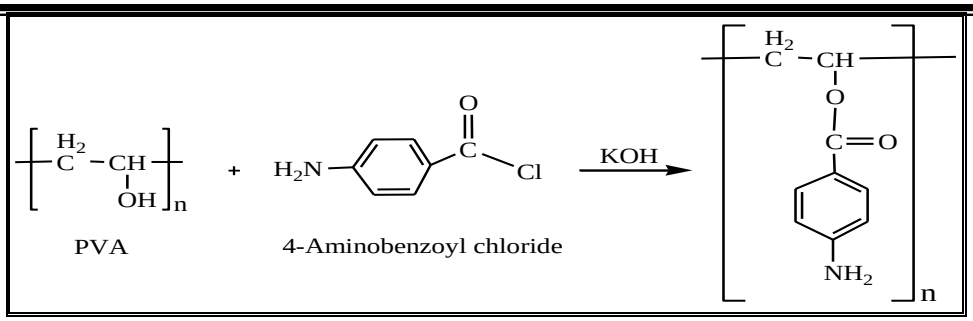
Evaluation testing of antimicrobial activity:

Antimicrobial susceptibility test measures the ability of an antimicrobial 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 ٥٠ 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 (10^5-10^6) Colony-Forming Units (CFU) per milliliter. After the culture has been incubated at ٣٧C° for ١٨ 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 **Results & Discussion**٧

PVAB was prepared from treatment of polyvinyl alcohol with ٤-aminobenzoyl chloride in presence of potassium hydroxide and DMSO as solvent with stirring for ٤٨ hours (scheme ١). The FTIR spectrum for compound (PVAB) exhibited the band at (٣٣٤٤) cm^{-1} due to (NH_2) and (١٦٨٨) cm^{-1} due to ($C=O_{est.}$) and disappearance the band at (٣٤١٥) cm^{-1} due to [٩, ١٠].

(OH) groups for PVA spectrum.



Scheme (١): Preparation of the poly (vinyl-٤-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 Gram–positive bacterial strains (*Staphylococcus aureus* and *Staphylococcus albus*) and two fungal (*Aspergillus Niger* and *Yeast*) Tables (١-٧) and (٨-١٤) respectively.

Table (١): Minimum inhibitory concentration (µg/ml) of (PVAB) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml											
		٥٥٠	٦٠٠	٦٥٠	٧٠٠	٧٥٠	٨٠٠	٨٥٠	٩٠٠	٩٥٠	١٠٠٠	١٠٥٠	١١٠٠
Escherichia Coli	**-ve**	+	+	+	+	+	-	-	-	-	-	-	-
Shigella dysentery	**-ve**	+	+	+	+	+	+	+	-	-	-	-	-
Klebsiella Pneumoniae	**-ve**	+	+	+	+	+	+	+	-	-	-	-	-
Staphylococcus aureus	**+ve**	+	+	+	+	+	+	+	+	-	-	-	-
Staphylococcus albus	**+ve**	+	+	+	+	+	+	+	+	+	+	-	-

Table (٢): Minimum inhibitory concentration (µg/ml) of (PVAB-Hg) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml											
		١٠	٢٠	٣٠	٤٠	٥٠	١٠٠	١٥٠	٢٠٠	٢٥٠	٣٠٠	٣٥٠	٤٠٠
Escherichia Coli	**-ve**	+	-	-	-	-	-	-	-	-	-	-	-
Shigella dysentery	**-ve**	+	-	-	-	-	-	-	-	-	-	-	-
Klebsiella Pneumoniae	**-ve**	+	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	**+ve**	+	+	-	-	-	-	-	-	-	-	-	-
Staphylococcus albus	**+ve**	+	+	-	-	-	-	-	-	-	-	-	-

Table (٣): Minimum inhibitory concentration (µg/ml) of (PVAB-Ag) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml											
		٥٠	١٠٠	١٥٠	٢٠٠	٢٥٠	٣٠٠	٣٥٠	٤٠٠	٤٥٠	٥٠٠	٥٥٠	٦٠٠
Escherichia Coli	-ve	+	-	-	-	-	-	-	-	-	-	-	-
Shigella dysentery	-ve	+	-	-	-	-	-	-	-	-	-	-	-
Klebsiella Pneumoniae	-ve	+	+	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	+ve	+	+	-	-	-	-	-	-	-	-	-	-
Staphylococcus albus	+ve	+	+	+	-	-	-	-	-	-	-	-	-

Table (٤): Minimum inhibitory concentration (µg/ml) of (PVAB-Cu) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml											
		١٥٠	٢٠٠	٢٥٠	٣٠٠	٣٥٠	٤٠٠	٤٥٠	٥٠٠	٥٥٠	٦٠٠	٦٥٠	٧٠٠
Escherichia Coli	-ve	+	-	-	-	-	-	-	-	-	-	-	-
Shigella dysentery	-ve	+	-	-	-	-	-	-	-	-	-	-	-
Klebsiella Pneumoniae	-ve	+	+	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	+ve	+	+	-	-	-	-	-	-	-	-	-	-
Staphylococcus albus	+ve	+	+	-	-	-	-	-	-	-	-	-	-

Table (٥): Minimum inhibitory concentration (µg/ml) of (PVAB-Co) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml											
		٤٥٠	٥٠٠	٥٥٠	٦٠٠	٦٥٠	٧٠٠	٧٥٠	٨٠٠	٨٥٠	٩٠٠	٩٥٠	١٠٠٠
Escherichia Coli	-ve	+	+	+	+	+	-	-	-	-	-	-	-
Shigella dysentery	-ve	+	+	+	+	+	+	-	-	-	-	-	-
Klebsiella Pneumoniae	-ve	+	+	+	+	+	+	-	-	-	-	-	-
Staphylococcus aureus	+ve	+	+	+	+	+	+	+	-	-	-	-	-
Staphylococcus albus	+ve	+	+	+	+	+	+	+	-	-	-	-	-

Table (٦): Minimum inhibitory concentration (µg/ml) of (PVAB-Ni) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml											
		٤٥٠	٥٠٠	٥٥٠	٦٠٠	٦٥٠	٧٠٠	٧٥٠	٨٠٠	٨٥٠	٩٠٠	٩٥٠	١٠٠٠
Escherichia Coli	-ve	+	+	+	+	+	-	-	-	-	-	-	-
Shigella dysentery	-ve	+	+	+	+	+	+	-	-	-	-	-	-
Klebsiella Pneumoniae	-ve	+	+	+	+	+	+	+	-	-	-	-	-
Staphylococcus aureus	+ve	+	+	+	+	+	+	+	+	-	-	-	-
Staphylococcus albus	+ve	+	+	+	+	+	+	+	+	-	-	-	-

Table (٥): Minimum inhibitory concentration (µg/ml) of (PVAB-Mn) against bacteria isolated.

Isolates	Gram Stain	Concentration µg/ml											
		٤٥٠	٥٠٠	٥٥٠	٦٠٠	٦٥٠	٧٠٠	٧٥٠	٨٠٠	٨٥٠	٩٠٠	٩٥٠	١٠٠٠
Escherichia Coli	-ve	+	+	+	+	+	+	+	-	-	-	-	-
Shigella dysentery	-ve	+	+	+	+	+	+	+	+	-	-	-	-
Klebsiella Pneumoniae	-ve	+	+	+	+	+	+	+	+	-	-	-	-
Staphylococcus aureus	+ve	+	+	+	+	+	+	+	+	-	-	-	-
Staphylococcus albus	+ve	+	+	+	+	+	+	+	+	+	+	-	-

Table (٨): Minimum inhibitory concentration (µg/ml) of (PVAB) against fungi isolated.

Isolates	Concentration µg/ml										
	١٠٠٠	١٠٥٠	١١٠٠	١١٥٠	١٢٠٠	١٢٥٠	١٣٠٠	١٣٥٠	١٤٠٠	١٤٥٠	١٥٠٠
Aspergillus Niger	+	+	+	+	+	+	+	+	-	-	-
Yeast	+	+	+	+	+	+	+	-	-	-	-

Table (٩): Minimum inhibitory concentration (µg/ml) of (PVAB-Hg) against fungi isolated.

Isolates	Concentration µg/ml										
	٧٥٠	٨٠٠	٨٥٠	٩٠٠	٩٥٠	١٠٠٠	١٠٥٠	١١٠٠	١١٥٠	١٢٠٠	١٢٥٠
Aspergillus niger	+	+	-	-	-	-	-	-	-	-	-
Yeast	+	+	+	-	-	-	-	-	-	-	-

Table (١٠): Minimum inhibitory concentration (µg/ml) of (PVAB-Ag) against fungi isolated.

Isolates	Concentration µg/ml										
	٧٥٠	٨٠٠	٨٥٠	٩٠٠	٩٥٠	١٠٠٠	١٠٥٠	١١٠٠	١١٥٠	١٢٠٠	١٢٥٠
Aspergillus niger	+	+	+	-	-	-	-	-	-	-	-
Yeast	+	+	+	+	+	-	-	-	-	-	-

Table (١١): Minimum inhibitory concentration (µg/ml) of (PVAB-Cu) against fungi isolated.

Isolates	Concentration µg/ml										
	٧٥٠	٨٠٠	٨٥٠	٩٠٠	٩٥٠	١٠٠٠	١٠٥٠	١١٠٠	١١٥٠	١٢٠٠	١٢٥٠
Aspergillus niger	+	+	+	-	-	-	-	-	-	-	-
Yeast	+	+	+	+	+	+	-	-	-	-	-

Table (١٢): Minimum inhibitory concentration (µg/ml) of (PVAB-Co) against fungi isolated.

Isolates	Concentration µg/ml										
	٨٥٠	٩٠٠	٩٥٠	١٠٠٠	١٠٥٠	١١٠٠	١١٥٠	١٢٠٠	١٢٥٠	١٣٠٠	١٣٥٠
Aspergillus niger	+	+	+	+	-	-	-	-	-	-	-
Yeast	+	+	+	+	+	+	-	-	-	-	-

Table (١٣): Minimum inhibitory concentration (µg/ml) of (PVAB-Ni) against fungi isolated.

Isolates	Concentration µg/ml										
	٨٥٠	٩٠٠	٩٥٠	١٠٠٠	١٠٥٠	١١٠٠	١١٥٠	١٢٠٠	١٢٥٠	١٣٠٠	١٣٥٠
Aspergillus niger	+	+	+	+	-	-	-	-	-	-	-
Yeast	+	+	+	+	+	-	-	-	-	-	-

Table (١٤): Minimum Inhibitory Concentration (µg/ml) of (PVAB-Mn) against fungi isolated.

Isolates	Concentration µg/ml										
	١٠٠٠	١٠٥٠	١١٠٠	١١٥٠	١٢٠٠	١٢٥٠	١٣٠٠	١٣٥٠	١٤٠٠	١٤٥٠	١٥٠٠

Aspergillus niger	+	+	+	+	+	+	+	-	-	-	-
Yeast	+	+	+	+	+	+	-	-	-	-	-

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 [١١]. 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 [١٢].

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 [١٣,١٤], 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 [١٥].

In the other side the presence of some compound in the microbial agent which have some groups like (-SH,-NH_2, -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 reasons causing enhancement of the bactericidal activity of the metal complexes as compared to the uncomplexed ester compounds [١٦,١٧].

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 and Ag) مع بولي (فاينيل-٤- بنزوات الامين)

الخلاصة

يتضمن هذا البحث تحضير بولي (فاينيل -٤- بنزوات الامين) من تفاعل بولي فاينيل الكحول مع (٤- 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, Shigella dysentery, Klebsiella pneumonae, Staphylococcus aureus, Staphylococcus Albus*) ونوعين من الفطريات (*Aspergillus niger, Yeast*). 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.