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Study of Suspended Solution from Blending Three Polymer Metal Complexes as Antimicrobial

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Abstract
In this study, different weight percent of poly(vinyl alcohol) (PVA)/poly(ethylene glycol) (PEG)/poly (acryl amide) (PAAm) blend solutions were prepared by solution blending followed by preparing of polymer metal complexes with Ag (I), Cu (II), Ni (II) and Hg (II). 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*). Polymer metal complexes showed different activities against the various microbial isolates. The polymer metal complexes showed higher activity than the free polymer.

Keywords: poly (vinyl alcohol), poly (ethylene glycol), poly (acryl amide), ternary blend polymers, antimicrobial activity, polymer metal complexes.

1. Introduction

Polymer blends, that is, physical mixture of structurally different polymers which interact with secondary forces such as hydrogen bonding with no covalent bonding. Polymer blends have been widely used in the industry because of their ability to combine in a unique material the properties of their components, at a relatively low cost when compared to the development of a new polymer. It is well-known that the properties of polymer blends are greatly influenced by the morphology that is developed during the mixing process.

Blending of three or more polymers has become an increasingly important technique for preparing materials with tailor made properties different from those of the constituent polymers. Blending of polymers may result in reducing their basic cost, improving their processing and maximizing their important properties. The increase in properties of the blend depends on the degree of compatibility or miscibility of polymers at the molecular level. (Mudigoudra et al 2012)

Polyethylene glycol (PEG) is finding a rapidly expanding use in biochemical and biomedical applications. It has been found to be non-toxic, non-immunogenic and water-soluble. PEG has therefore been used in protein modification to decrease antigenicity, prolong its plasma circulatory half-life and to increase its solubility and thermal stability (Matsushima et al 1980),(Ashihara et al 1978),(Abuchowski et al 1977), (Bariyanga, J. 2002).

While poly acryl amide has found numerous applications as a soil conditioner, in wastewater treatment, in the cosmetic, paper, and textile industries, and in the laboratory as a solid support for the separation of proteins by electrophoresis (Friedman 2003).

Poly (vinyl alcohol), henceforth referred to as PVA, has become a prime candidate for improved biomaterials and drug delivery systems. PVA is a relatively inert polymer which is easily process able. PVA is hydrophilic and therefore swells in the presence of water or biological fluids to form hydrogels. This property is particularly useful because it can allow for the release of drugs incorporated into these hydrogels. Other polymers, such as poly (acrylic acid) (PAA) and poly(ethylene glycol) (PEG), can be blended with PVA to impart additional properties such as pH sensitivity or improved blood response (Friedman 2003), (Gudeman& Peppas 1995).

In this study was to assess the antimicrobial action of mixtures (polyvinyl alcohol, polyethylene glycol and poly acryl amide) of different concentrations and different metals Cu, Ni, Ag, Hg. Two methods were used, first by preparing polymeric complexes for each polymer and then blended in different proportions and studied; the second method involved mixing ratios of these polymers and then prepares complexes and studied. The minimum inhibitory concentration (MIC) was determined by using the agar dilution method against.

Experimental

Materials

Poly Vinyl Alcohol (PVA) (Mw = 145000), Polyethylene glycol (PEG) (Mw=8000) and Poly acryl amide (PAA) (10000) was purchased from Aldrich and Merck.

1-Preparation of standard solution polymers

The standard solutions of (Polyvinyl alcohol (A), Polyethylene glycol (B) and Poly acryl amide(C) were prepared by taking 5 gm of (each polymer) with 100 ml of deionizer water.

2-preparation of standard solution metals

The metal agent's solutions were prepared by taking ($CuCl_2.2H_2O$ 0.05 gm., $AgNO_3$ 0.0676 gm., $NiCl_2.2H_2O$ 0.0884 gm., $HgCl_2$ 0.082 gm.) with 20 ml of deionizer water .we obtained four standard solutions (Cu), (Ag), (Ni), (Hg).

3-Preparation of compounds studied as an anti-bacterial

A-preparation of standard solution from polymers complexes:

The polymer standard solution (A-Cu) was prepared by addition of standard solution (A) to the metals standard solution (Cu) (w 50%-w50%) mixture & it was stirred for 1 hr. the polymer standard solutions (A-Ag), (A-Ni), (A-Hg) were prepared in the same way. The polymers standard solution (B-Cu), (B-Ag), (B-Ni), (B-Hg), (C-Cu), (C-Ag), (C-Ni), (C-Hg) were prepared in the same way above. The polymers standard solution prepared above were mixed in certain ratio as in the tables (1).

Table (1): preparation of standard solution from polymers complexes

Sample No.	Polymer A	Polymer B	Polymer C
Sample 1	50% A-Cu	25% B-Cu	25% C-Cu
Sample 2	25% A-Cu	50% B-Cu	25% C-Cu
Sample 3	25% A-Cu	25% B-Cu	50% C-Cu
Sample 4	100% A-Cu	-	-
Sample 5	-	100% B-Cu	-
Sample 6	-	-	100% C-Cu
Sample 7	50% A-Ag	25% B-Ag	25% C-Ag
Sample 8	25% A-Ag	50% B-Ag	25% C-Ag
Sample 9	25% A-Ag	25% B-Ag	50% C-Ag
Sample 10	100% A-Ag	-	-
Sample 11	-	100% B-Ag	-
Sample 12	-	-	100% C-Ag
Sample 13	50% A-Ni	25% B-Ni	25% C-Ni
Sample 14	25% A-Ni	50% B-Ni	25% C-Ni
Sample 15	25% A-Ni	25% B-Ni	50% C-Ni
Sample 16	100% A-Ni	-	-
Sample 17	-	%100 B-Ni	-
Sample 18	-	-	100% C-Ni
Sample 19	50% A-Hg	25% B-Hg	25% C-Hg
Sample 20	25% A-Hg	50% B-Hg	25% C-Hg
Sample 21	25% A-Hg	25% B-Hg	50% C-Hg
Sample 22	100% A-Hg	-	-
Sample 23	-	100% B-Hg	-
Sample 24	-	-	100% C-Hg

B-preparation of standard solution from mixture of polymers with metals:
Standard mixed Solution of the polymers were prepared by mixing in certain ratio from standard solution polymer A,B and C with stirring to obtain standards A2BC , AB2C, ABC2 as the table (2), these standard mixed Solution of polymers were left for 24 hr.

Table (2): preparation of standard solution from mixture of polymers with metals

A2BC	50% A	25% B	25% C
AB2C	25% A	50% B	25% C
ABC2	25% A	25% B	50% C

The standards solution of mixed polymers A2BC , AB2C, ABC2 prepared above mixed in equal ratio with standard solutions (Cu), (Ag),(Ni),(Hg) as the table (3):

Table (3): mixing the standards solution of mixed polymers with equal ratio of standard solutions metals.

Sample No.	Polymer mixing solution	Metal solution
Sample 25	50% A2BC	50% Cu
Sample 26	50% A2BC	50% Ag
Sample 27	50% A2BC	50% Ni
Sample 28	50% A2BC	50% Hg
Sample 29	50% A2BC	50% deionizer water
Sample 30	50% AB2C	50% Cu
Sample 31	50% AB2C	50% Ag
Sample 32	50% AB2C	50% Ni
Sample 33	50% AB2C	50% Hg
Sample 34	50% AB2C	50% deionizer water
Sample 35	50% ABC2	50% Cu
Sample 36	50% ABC2	50% Ag
Sample 37	50% ABC2	50% Ni
Sample 38	50% ABC2	50% Hg
Sample 39	50% ABC2	50% deionizer water

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* 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 (Julio 1982),(Collee et al 1999).

Results& Discussion:

In this study, the use of two types of physical mixture of polymers, first by preparing polymeric complexes for each polymer and then blended in different proportions and studied, the second method involved mixing ratios of these polymers and then prepare complexes and studied , the antimicrobial activity of the blend polymer metal complexes in this two types of physical mixture was determined against three Gram-negative bacterial strains (*Escherichia coli,Shigella dysenteryand KlebsiellaPneumoniae*), two Gram–positive bacterial strains (*Staphylococcus aureus and Staphylococcus albus*) and two fungal (*Aspergillusniger and Yeast*) Tables (4) and (5) respectively.

Through tables note we are when you use a polymer metal complexes as an antimicrobial is better than the use of polymer this is due to the complexes Polymer metal showed higher activity than the free metal, 1al 1992).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(Andres 1981). 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. 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 (Rasmia et al 2014).

All polymer metal complexes in the first way showed higher activities against the various microbial isolates than the polymer metal complexes in the second method, because when we prepare polymer metal complexes and then mixed together in different proportions, it leads to the formation of polymer metal complexes well as be the function groups in polymers such as (-OH, NH2) is free. But the preparation of the blend polymers metal complexes by mixing polymers at different rates and then mixed with the elements, this will lead to increasing intramolecular between the active groups in these polymers and thus will reduce the preparation of polymeric complexes . The Poly acryl amide complexes exhibited a good degree of inhibitory effects on the growth of different bacterial better than poly vinyl alcohol complexes and polyethylene glycol complexes and the order of increasing activities was polymer polymer-Ni < polymer-Cu < polymer-Hg < polymer-Ag. 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.

Table (4): Minimum Inhibitory Concentration (µg/ml) of polymer metal complexes against *Isolated Bacteria gm-ve and gm +ve*

Polymer Complex	Isolates				
	Escherichia Coli (gm −ve)	*Shigella dysentery* (gm −ve)	*Klebsiella Pneumoniae* (gm −ve)	*Staphylococcus aureus* (gm +ve)	*Staphylococcus albus* (gm +ve)
Sample 1	400	450	300	450	450
Sample 2	350	400	350	500	300
Sample 3	300	300	400	300	300
Sample 4	800	850	700	800	700
Sample 5	650	700	750	750	650
Sample 6	500	500	500	500	550
Sample 7	400	500	450	450	450
Sample 8	350	350	300	400	350
Sample 9	260	350	250	250	350
Sample 10	600	650	700	500	700
Sample 11	550	500	600	550	550
Sample 12	450	450	600	500	500
Sample 13	400	500	400	400	400
Sample 14	400	400	450	500	400
Sample 15	300	350	400	350	350
Sample 16	800	850	650	900	700
Sample 17	850	950	750	850	850
Sample 18	600	650	700	650	650
Sample 19	400	450	400	350	500
Sample 20	500	400	600	600	500
Sample 21	350	350	450	350	300
Sample 22	700	800	800	750	700
Sample 23	800	850	800	800	750
Sample 24	550	600	500	600	550
Sample 25	900	1000	900	950	1000
Sample 26	800	800	700	800	850
Sample 27	850	900	900	900	900
Sample 28	850	850	850	850	950
Sample 29	900	950	950	950	900
Sample 30	900	900	900	900	950
Sample 31	850	950	950	950	850
Sample 32	900	900	850	900	950
Sample 33	900	900	850	900	950
Sample 34	950	1000	1050	850	950
Sample 35	850	850	850	900	900
Sample 36	800	900	900	850	800
Sample 37	850	850	850	850	900
Sample 38	800	900	800	800	800
Sample 39	900	900	900	1000	950
Sample 40	1100	1100	950	1100	1150
Sample 41	1050	1100	1050	1100	1000
Sample 42	1050	1000	1050	1050	1000

concentration compounds metal µg/ml

Table (5) Minimum Inhibitory Concentration (µg/ml) of polymer metal complexes against *Isolated fungal*

Concentration compounds metal µg/ml	Polymer complex	Isolates	
		Aspergillus niger.	*Yeast*
	Sample 1	800	850
	Sample 2	700	700
	Sample 3	750	650
	Sample 4	1200	1150
	Sample 5	1050	1100
	Sample 6	950	900
	Sample 7	800	800
	Sample 8	700	750
	Sample 9	650	600
	Sample 10	1050	1100
	Sample 11	900	900
	Sample 12	800	900
	Sample 13	800	750
	Sample 14	800	750
	Sample 15	650	650
	Sample 16	1050	1150
	Sample 17	1250	1150
	Sample 18	1000	1000
	Sample 19	800	750
	Sample 20	900	850
	Sample 21	750	750
	Sample 22	950	1100
	Sample 23	1150	1150
	Sample 24	950	950
	Sample 25	1150	1250
	Sample 26	1150	1150
	Sample 27	1200	1250
	Sample 28	1200	1200
	Sample 29	1200	1250
	Sample 30	1200	1250
	Sample 31	1200	1150
	Sample 32	1300	1350
	Sample 33	1250	1250
	Sample 34	1300	1350
	Sample 35	1250	1200
	Sample 36	1200	1150
	Sample 37	1250	1300
	Sample 38	1150	1150
	Sample 39	1250	1250
	Sample 40	1500	1450
	Sample 41	1400	1350
	Sample 42	1350	1400

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