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Coordination Chemistry of Cu(II) with Polyvinyl alcohol(PVA) and Some Amino acids and DNA.

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***Abstract*—The acid-base equilibria of polyvinyl alcohol(PVA) is investigated. The stability constant values of the binary and ternary complexes formed in solution between polyvinyl alcohol, Cu(II), some amino acids and DNA were determined potentiomertically. The stability constants of the complexes are determined and the concentration distribution diagrams of the complexes are evaluated. The ligand and their metal chelates have been screened for their antimicrobial activities using the disc diffusion method against the selected bacteria and fungi. Binary and ternary complexes of copper(II) involving polyvinyl alcohol(PVA) and various biologically relevant ligands containing different functional groups, were investigated. The ligands (L) are amino acids and DNA constituents. The ternary complexes of amino acids and DNA are formed by simultaneous and stepwise processes respectively. The results showed the formation of Cu(PVA)(L) complexes with amino acids and DNA. Amino acids form both Cu(PVA)(L) complexes and the corresponding protonated Cu(PVA)(LH*)* and deprotonated species Cu(PVA)(LH_{-1}*)*. The ternary complexes of copper(II) with (PVA) and DNA are formed in a stepwise process, whereby binding of copper(II) to (PVA) is followed by ligation of the DNA components. DNA constituents form 1:1 complexes with Cu(PVA). The concentration distribution of various complex species formed in solution was also evaluated as a function of pH.**

***Keywords*—Copper(II), Amino acids, DNA constituents, Stability constant, Spectroscopic characterization, Biological activity**

I. Introduction

Poly(vinyl alcohol) (PVA; -[-CH-CHOH-]n-) is the world's largest volume synthetic polymer produced for its excellent chemical resistance and physical properties and complete biodegradability, which has led to broad practical applications. PVA is a semi-crystalline polymer whose crystalline index depends on synthetic process and the physical aging [1,2]. PVA is a water-soluble polyhydroxy polymer, one of the few linear, non-halogenated aliphatic polymers. PVA has a two dimensional hydrogen-bonded network sheet structure. PVA is an important material in view of its large scale applications. It is used in surgical devices, sutures, hybrid islet transplantation, implantation, blend membrane and in synthetic cartilage in reconstructive joint surgery [3-6]. In this investigation we report a quantitative study of the acid base equilibrium of PVA, as well as the binary complex formation equilibria with Cu(II). Mixed ligand complexes involving Cu-PVA and some amino acids and DNA constituents are studied and the concentration distribution of various complex species formed in solution was also evaluated.

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II. Experimental

A. Materials and reagents

PVA (average M_W: 27000, Aldrich makes). Glycine, alanine, threonine, methionine, proline, serine, phenylalanine, lysine, imidazole, L-histidine·HCl, and L-histamine·2HCl, were provided by the Sigma Chem. Co. The DNA constituents uracil, uridine, thymine, thymidine, inosine, inosine 5′-monophosphate and adenosine 5′-monophosphate were supplied by BDH-Biochemicals Ltd. For stability constant determination, solutions of (PVA) were prepared in deionized water , freshly prepared solutions of (PVA) were used for all the measurements. L-histidine·HCl was prepared in one equivalent of HNO_3 acid, $Cu(NO_3)_2 \cdot 2H_2O$ was provided by BDH. The copper content of the solutions was determined by complexometric EDTA titrations [7].

B. Biological activity

The metal complexes were evaluated for their antibacterial activity against Staphylococcus aureus (Gram-positive bacteria), Escherichia coli (Gram-negative bacteria) and also for their antifungal activity against Candida albicans and Aspergillus flavus using the disc diffusion technique as described in British Pharmacopoeia (2000). Nutrient agar was melted at 45.00 °C, and inoculated with the cell suspension (1:100) bacteria or yeast. The flask was shaken well and poured into a Petri-dish (15 cm in diameter). Filter paper discs (6 mm) Whatman No. 2 were thoroughly moistened with antibiotics (50 g) the treated discs were aseptically transferred and placed on the surface of the inoculated plates and kept in a refrigerator for 1 h to permit diffusion of antimicrobial substances. The plates were incubated at 37.00 °C, for 24 h in the case of bacteria and at 28.00 °C, for 48 h in the case of fungi. The zones of inhibition were measured in mm. The mean values of inhibition were calculated from triple reading in each test [8,9]. The antibacterial and antifungal activities are calculated as a mean of three replicates. The antibacterial and antifungal activity of a common standard antibiotic *Ampicillin* and *Amphotericine B* were also recorded using the same protocol and at the same concentration and solvent. The antibacterial and antifungal results of the complexes were compared with the standard and the % activity index (A.I) for the M(II) complexes were calculated by using the formula:

$$A.I = Z_{\tau\chi} / Z_{\sigma} \quad \xi\, 100 \qquad (1)$$

Z_{tc}: Zone of inhibition by test complexes (diameter)
Z_s: Zone of inhibition by standard (diameter)

C. Apparatus and measuring techniques

Potentiometric measurements were made using a Metrohm 751 Titrino. The titroprosessor and electrode were calibrated with standard buffer solutions, prepared according to NBS specifications[10] at 25 ± 0.1°C and I = 0.1 mol dm^{-3}, potassium hydrogen phthalate (pH 4.008) and a mixture of KH_2PO_4 and Na_2HPO_4 (pH 6.865). A (0.10 mol dm^{-3}) standard acid solution was titrated with a standard base (0.10 mol dm^{-3}) to convert the pH meter reading into hydrogen ion concentration. The pH values was plotted against p[H], where the relation pH- p[H] =0.5 was observed for all the titration data. A pK_w value of 13.997[11] was used to calculate the $[OH^-]$. The titrations were performed in a thermostated titration vessel equipped with a magnetic stirring system, under purified N_2 atmosphere using 0.05 M NaOH as titrant. The titrations were performed at a constant ionic strength of 0.1 mol. dm^{-3} ($NaNO_3$). The acid dissociation constants of the ligand were determined by titrating a 40 ml of ligand solution (1.25×10^{-3} mol dm^{-3}). The formation constants of the complexes were determined by titrating 40 mL of the solution containing metal ion (1.25×10^{-4} mol dm^{-3}) and ligand (1.25×10^{-3} mol dm^{-3}). The stability constant values were calculated by using the computer program MINIQUAD-75[12]. Various possible composition models were tried to calculate the stoichiometry and stability constants of the system studied. The model selected was that which gave the best statistical fit as described before [12]. Table 1 lists the stability constants together with their standard deviations and the sum of the squares of the residuals derived from the MINIQUAD output. The speciation diagrams were obtained using the program SPECIES [13]. UV-Vis spectrophotometric measurements were carried out using automated spectrophotometer UV-Vis Thermo Fischer Scientific Model Evolution 60 ranged from 200 to 900 nm.

D. Equilibrium Measurements

The acid dissociation constants of the ligands were determined potentiometrically by titrating the ligand (40 cm^3) solution (1.25×10^{-3} mol·dm^{-3}) of constant ionic strength 0.1 mol·dm (adjusted with $NaNO_3$). The stability constant of the Cu(PVA) complex was determined by titrating 40 cm^3 of a solution mixture of Cu^{II} (1.25×10^{-3} mol·dm^{-3}), PVA ligand (2.5×10^{-3} mol·dm^{-3}) and $NaNO_3$ (0.1 mol·dm^{-3}). The formation constant of the mixed ligand complexes were determined by titrating solution mixtures containing equivalent amounts of Cu^{II} (1.25×10^{-3} cm^3), PVA and other ligands in the concentration ratio 1:1:1 for amino acids and the DNA constituents.

All titrations were performed in a purified N_2 atmosphere using aqueous 0.05 mol·dm^{-3} NaOH as titrant. The general four component equilibrium can be written as follows (charges are omitted for simplicity)

$$\beta_{lpqr} = [(Cu)_l (Prom)_p (L)_q (H)_r] / [(Cu)_l (Prom)_p (L)_q (H)_r] \quad (2)$$

E. Spectrophotometric Measurements

Spectrophotometric investigation of the binary and ternary complexes was performed by scanning the visible spectra of solution mixtures 1–6. Under the experimental conditions and after neutralization of the hydrogen ions released, it is assumed that the complexes have been completely formed. Mixtures 1–6 were prepared to be used for spectrophotometric measurements. In each case the volume is made up to 10 cm^3 by water, and the ionic strength is kept constant by using $NaNO_3$ (0.1 mol·dm^{-3})

1. 1.0 cm^3 Cu(II) (0.01 mol·dm^{-3}) + 1.25 cm^3 $NaNO_3$ (0.80 mol·dm^{-3})
2. 1.0 cm^3 glycine or thymidine (0.01 mol·dm^{-3}) + 1.25 cm^3 $NaNO_3$ (0.8 mol·dm^{-3})
3. 1.0 cm^3 PVA (0.01 mol·dm^{-3}) + 1.25 cm^3 $NaNO_3$ (0.8 mol·dm^{-3})
4. 1.0 cm^3 Cu(II) (0.01 mol·dm^{-3}) + 1.0 cm^3 PVA (0.01 mol·dm^{-3}) + 1.25 cm^3 $NaNO_3$ (0.8 mol·dm^{-3})
5. 1.0 cm^3 Cu(II) (0.01 mol·dm^{-3}) + 1.0 cm^3 glycine or thymidine (0.01 mol·dm^{-3})+1.25 cm^3 $NaNO_3$ (0.8 mol·dm^{-3})
6. 1.0 cm^3 Cu(II) (0.01 mol·dm^{-3}) + 1.0 cm^3 PVA (0.01 mol·dm^{-3}) + 1.0 cm^3 glycine or thymidine (0.01 mol·dm^{-3}) + 1.25 cm^3 $NaNO_3$ (0.8 mol·dm^{-3})

III. Results and Discussion

The acid dissociation constants of the PVA and the formation constants of their binary complexes presented in Table 1, the potentiometric equilibrium titration curve of acid bade of PVA, Fig. 1, shows the presence of one buffer region in which the hydroxyl group is neutralized (pKa = 10.89 at 25^0C), yielding the anionic species PVA^{-1}, which starts to form above pH= 4 reaching its maximum concentration of ≈ 90 at pH, the stability of binary complex of Cu(II) and different amino acids were studied applying potentiometric measurements. The stability constants of binary complexes are allotted in Table 1, the concentration distribution of Cu- glycine and Cu-Threonine as representative curves occur in Fig. 2,3 the curve of binary complexes were significantly lower than PVA titration curve, this corresponds to the formation of a complex through release of a proton from a hydroxyl group. The experimental studies determined under the same experimental conditions of ionic strength and temperature used to study the Cu(II)–PVA and the corresponding ternary complexes. The results obtained are in good agreement with the literature data [14-16]

A. Ternary Complex Formation

Ternary complex formation may proceed either through a stepwise or a simultaneous mechanism depending on the chelating potential of polyvinyl alcohol and other ligands. The formation constants of the 1:1 copper(II) complexes with PVA and those of amino acids and DNA, taken from the literature and cited in Tables 1 and 2 are nearly of the same order. Consequently the ligation of PVA and amino acids will proceed simultaneously. The validity of this model was verified, by comparing the potentiometric data with the theoretically calculated (simulated) presents such a comparison, taken as a representative one. Figures 1 and 4 presents the potentiometric titration curve for the Cu–PVA–glycine system and Cu–PVA–inosine system according to the simultaneous mechanism.

1) *Complexes Involving Amino Acids*

The titration data of the ternary complexes with amino acids and PVA fit satisfactorily with formation of the species: $Cu(PVA)^+$, $Cu(PVA)_2$, $Cu(L)$, $Cu(L)_2$, $Cu(PVA)(L)$, $Cu(PVA)(L-1)$and $Cu(PVA)(LH)$. Ornithine (H_2L) is an α-amino acid having an extra amino group which may be protonated. Consequently the protonated ternary complex is detected. The coordination can be explained on the premise that ornithine is bound to $Cu(PVA)^+$ by the amino and carboxylic groups as glycine like, leaving the other amino group susceptible to protonation. The species distribution of glycine and threonine, taken as a representative amino acids, is given in Figs. 2 and 3. The protonated species 1111 in case of glycine complex is attains a maximum concentration at 57.5% at pH from 2-3, while in threonine this species is occur in 34% at pH. The deprotonated species 111-1 complex predominates, amounting to 99% in the physiological pH range 9-10, while the deprotonated species 1110 attains a maximum concentration of 71% at pH ≈ 6.5. Species with concentrations less than 5% were neglected in the concentration distribution plot for clarity. Threonine has an extra binding center on the β-alcohol-group. This group was reported to participate in complex formation [17]. The p*K*a value of the β-alcoholato-group incorporated in the Cu(II) complex ($\log_{10}\beta_{1110} - \log_{10}\beta_{111-1}$) is 7.68. This is in good agreement with that reported in literature for the Cu–threonine complex [18]. Charge effects will also be important since the alcohol is neutral, whereas the carboxylate group is negatively charged. Due to the donation of the electron pair on oxygen to the metal center, the OH bond can be considerably weakened and the ionization of a proton occurs at a lower pH. Histidine is a tridentate ligand having, amino, imidazole. and carboxylate groups as binding sites. With Cu(PVA), only two of the three binding sites are involved in complex formation. Hence, histidine coordinates in either a glycine-like or histamine-like nitration of % at pH mode. The stability constant values of histidine and histamine are of the same order and considerably higher than those of amino acids, which indicates that both histidine and histamine will preferably coordinate through the amino and imidazole groups

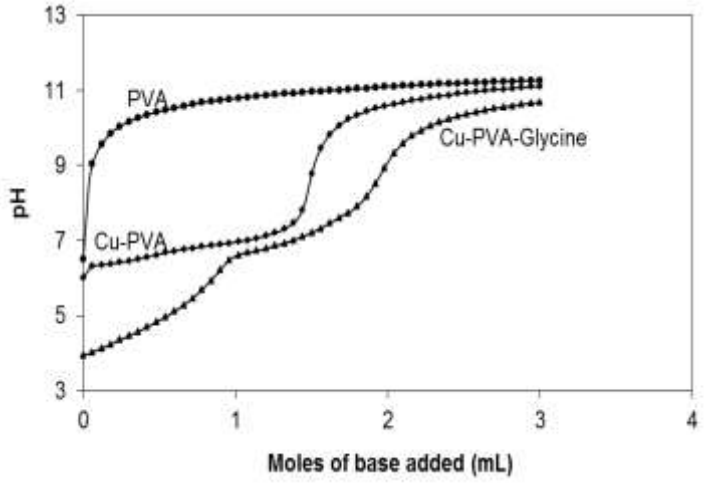

Figure 1. Potentiometric titration curve in the [Cu–PVA]–glycine system

TABLE 1 Formation Constants of the Binary and Ternary Complexes in the Cu^{ii} PVA Amino Acids at 25^0C and 0.1 M Ionic Strength

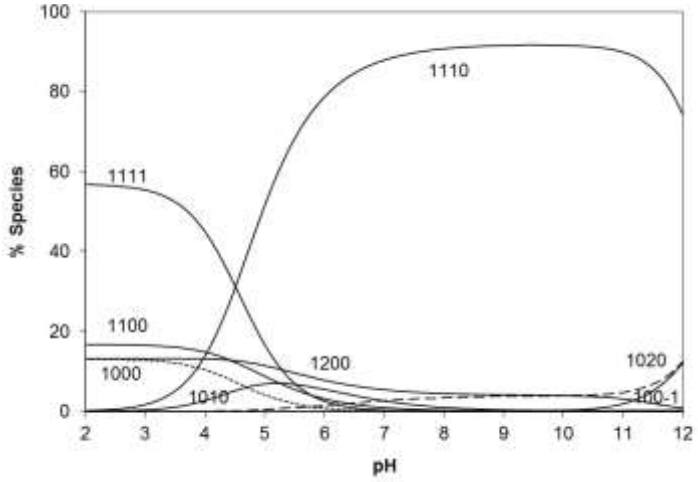

Figure 2. Concentration distribution of various species as a function of pH in the [Cu–PVA–glycine] system (each at a concentration of 1.25 mmol·L^{-1})

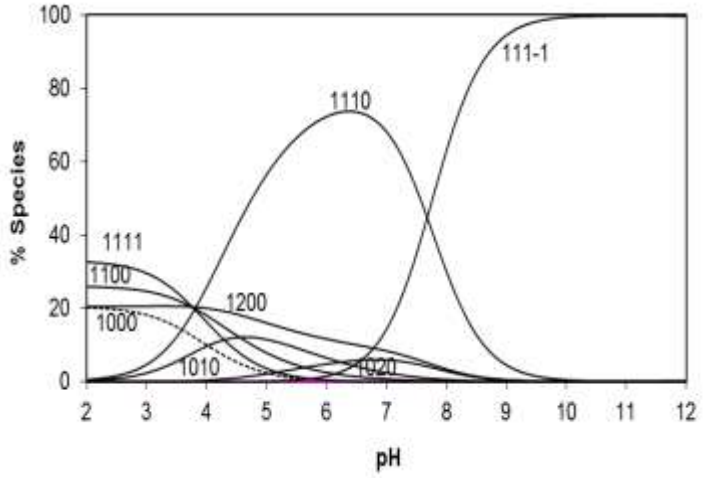

Figure 3. Concentration distribution of various species as a function of pH in the [Cu–PVA–threonine] system (each at a concentration of 1.25 mmol·L^{-1})

Cont. TABLE 1 Formation Constants of the Binary and Ternary Complexes in the Cu^{II} PVA Amino Acids at 25^0C and 0.1 M Ionic Strength

System	l	p	q	r[a]	$Log_{10}\beta$[b]	pK_a[c]	$Sd/10^{-8}$
$[Cu(H_2O)_4]^{2+}$	1	0	0	-1	-6.44(0.07)		
	1	0	0	-2	12.99(0.02)		$9.6x10^{-7}$
Cu-PVA	1	1	0	0	8.07(0.01)		$1.7x10^{-8}$
	1	2	0	0	15.93(0.03)		
Glycine	0	0	1	1	9.60(0.01)	9.60	$1.6x10^{-7}$
	0	0	1	2	11.93(0.02)	2.33	
	1	0	1	0	8.26(0.07)		$3.0x10^{-7}$
	1	0	2	0	13.57(0.09)		
	1	0	1	-1	0.34(0.08)		
	1	0	1	-2	10.07(0.06)		
	1	1	1	0	16.81(0.01)		$4.0x10^{-8}$
	1	1	1	1	21.32(0.01)	4.51	
Alanine	0	0	1	1	9.69(0.01)	9.69	$9.3x10^{-8}$
	0	0	1	2	11.89(0.02)	2.20	
	1	0	1	0	7.99(0.03)		$3.1x10^{-8}$
	1	0	2	0	14.18(0.02)		
	1	0	1	-1	-0.03(0.05)		
	1	0	1	-2	-9.30(0.01)		
	1	1	1	0	16.45(0.02)		$1.4x10^{-7}$
	1	1	1	1	22.00(0.03)	5.55	
B-phenyl-alanine	0	0	1	1	9.12(0.01)	9.12	$2.0x10^{-8}$
	0	0	1	2	11.01(0.03)	1.89	
	1	0	1	0	7.53(0.04)		$4.3x10^{-8}$
	1	0	2	0	13.73(0.03)		
	1	0	1	-1	0.001(0.05)		
	1	0	1	-2	-10.08(03)		
	1	1	1	0	16.62(0.02)		$1.6x10^{-7}$
	1	1	1	1	20.89(0.03)	4.17	
Proline	0	0	1	1	10.52(0.01)	10.52	
	0	0	1	2	12.03(0.04)	1.51	
	1	0	1	0	8.60(0.03)		
	1	0	2	0	15.09(0.01)		
	1	0	1	-1	1.29(0.04)		
	1	1	1	0	17.40(0.03)		
	1	1	1	1	22.18(0.02)	4.78	
Serine	0	0	1	1	9.14(0.02)	9.14	
	0	0	1	2	11.40(0.01)	2.26	
	1	0	1	0	8.4(90.02)		
	1	0	2	0	14.66(0.01)		
	0	0	1	-1	0.23(0.02)		
	1	1	1	0	15.56(0.02)		
	1	1	1	-1	8.59(0.03)		
Lysine	0	0	1	1	10.44(0.02)	10.44	
	0	0	1	2	19.66(0.02)	9.22	
	1	0	1	0	10.81(0.02)		
	1	0	2	0	18.22(0.02)		
	1	1	1	0	19.22(0.02)		
	1	1	1	1	26.09(0.02)	6.87	
Methionine	0 0	0 0	1 1	1 2	9.13(0.04) 11.10(0.02)	9.13 1.97	$1.6x10^{-7}$
	1	0	1	0	8.02(0.04)		
	1	0	2	0	14.79(0.09)		$1.6x10^{-7}$
	1	0	1	-1	0.78(0.06)		
	1	1	1	1	20.93(0.06)	4.80	

[a] *l*,*p*, *q* and *r* are the stoichiometric coefficients corresponding to Cu^{II}, PVA, amino acids and H^+ respectively;
the coefficient −1 refers to a proton loss
[b] $\log_{10}\beta$ of Cu–PVA–amino acids. Standard deviation are given in parentheses
[c] The pK_a of the ligands and the protonated species
[d] Sum of squares of residuals

Cont. TABLE 1 Formation Constants of the Binary and Ternary Complexes in the Cu^{ii} PVA Amino Acids at 25^0C and 0.1 M Ionic Strength

System	l	p	q	r[a]	$Log_{10}\beta$[b]	pK_a[c]	$Sd/10^{-8}$
Imidazole	0	0	1	1	7.04(0.01)	7.04	$1.6x10^{-7}$
	1	0	1	0	4.20(0.01)		
	1	0	2	0	7.62(0.04)		$1.6x10^{-7}$
	1	0	3	0	10.35(0.05)		
	1	1	1	0	16.25(0.08)		$1.6x10^{-7}$
	1	1	1	1	25.15(0.03)	8.90	
Threonine	0	0	1	1	9.11(0.01)	9.11	$7.0x10^{-8}$
	0	0	1	2	11.32(0.02)	2.21	
	1	0	1	0	7.97(0.03)		$3.1x10^{-8}$
	1	0	2	0	14.13(0.04)		
	1	0	1	-1	0.90(0.05)		
	1	0	1	-2	-8.93(0.02)		
	1	1	1	0	16.43(0.02)		$1.6x10^{-7}$
	1	1	1	1	20.23(0.07)	3.80	
	1	1	1	-1	8.75(0.06)	7.68	
Histidine	0	0	1	1	9.53(0.01)	9.53	$1.8x10^{-7}$
	0	0	1	2	15.81(0.02)	6.28	
	1	0	1	0	11.48(0.01)		$1.6x10^{-7}$
	1	0	2	0	19.70(0.06)		
	1	0	1	1	15.75(0.04)	4.27	
	1	0	1	-1	6.82(0.03)		
	1	0	1	-2	-3.77(0.04)		
	1	1	1	0	17.53(0.05)		$1.6x10^{-7}$
	1	1	1	1	22.36(0.05)	4.83	
Histamine	0	0	1	1	9.88(0.01)	9.88	$1.6x10^{-7}$
	0	0	1	2	15.97(0.01)	6.09	
	1	0	1	0	10.20(0.01)		$1.6x10^{-7}$
	1	0	2	0	17.53(0.03)		
	1	0	1	1	14.89(0.01)	4.69	
	1	0	1	-1	3.48(0.04)		
	1	0	1	-2	-5.86(0.02)		$1.6x10^{-7}$
	1	1	1	0	19.54(0.02)		
	1	1	1	1	24.00(0.02)	4.46	

Complexes Involving DNA-Unit Constituents

In the ternary complexes of DNA constituents, D, the potentiometric titration curves of the mixed ligand system (Fig. 4) could be fitted concerning the formation of the ternary complexes with stoichiometric coefficient 110, 111 and 120. In this respect, The Cu^{II}–PVA complex is first formed due to its greater stability compared to the Cu^{II}–DNA complex (Table 2). Beyond a = 2, the formation of the ternary complex was ascertained by comparison of the mixed ligand titration curve with the composite curve obtained by graphical addition of PVA titration data to that of the Cu^{II}–inosine titration curve. The mixed ligand system was found to deviate considerably from the resulting composite curve, indicating the formation of a ternary complex. Thus, the following equilibria can be written to describe the formation of the ternary complex (charges are omitted for simplicity) (Eqs. 3 and 4)

$$Cu + PVA \leftrightarrow Cu(PVA) \quad (3)$$
$$Cu(PVA) + D \leftrightarrow Cu(PVA)D \quad (4)$$

The pyrimidinic species (uracil, uridine thymine and thymidine) have only basic nitrogen donor atoms (N_3–C_4O group) in the measurable pH range and as a consequence they form 1:1 complexes with the Cu(PVA) ion. The thymidine complex is more stable than that with uridine, most probably owing to the higher basisity of the N_3 of thymidine, resulting from the inductive effect of the extra electron-donating methyl group. As a result of the high p*K*a values of pyrimidines (p*K*a > 9*)* and the fact that they are monodentate, the complexes are formed only above pH = 6, supporting the view that the negatively charged nitrogen donors of pyrimidine bases are important binding sites in the neutral and slightly basic pH ranges.

Mixed ligand complexes of nucleosides are less stable than the corresponding bases as is evident from the stability constants given in Table 4. The presence of sugar residue imposes steric hindrance in nucleosides for their complexation with metal ions and reduces the overall basicity of metal complexes of nucleosides considerably. The purines inosine and inosine 5′-monophosphate (IMP) have two metal ion binding centers: the N_1 and N_7 nitrogens. The results showed that inosine forms the complexes 110 and 111, while inosine 5′-monophosphate forms 110, 111 and 112 complexes. Inosine-5′-monophosphate (IMP) forms a more stable complex with Cu(PVA) than that with inosine. The extra stabilization can be attributed to the triply negatively charged 5′-IMP^{3-} ion.

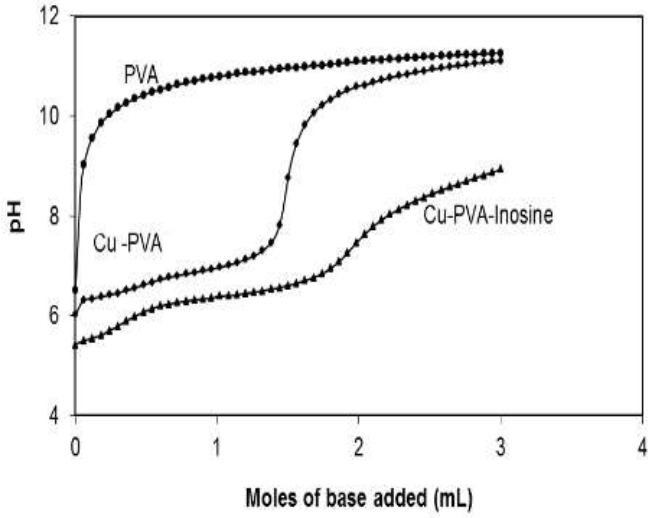

Figure 4. Potentiometric titration curve in the [Cu–PVA]–inosine system

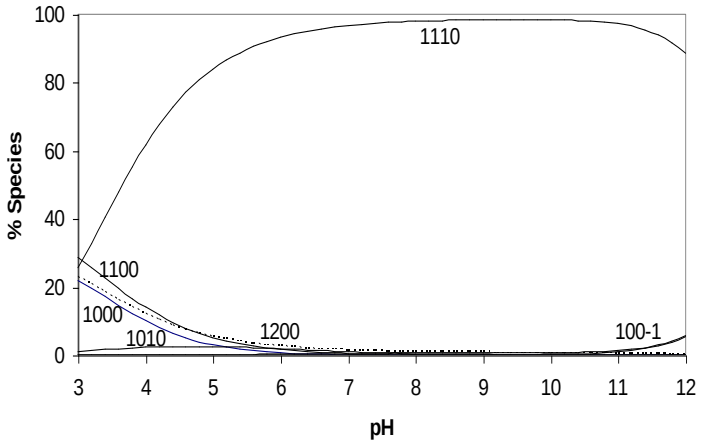

Figure 6. Concentration distribution of various species as a function of pH in the [Cu–PVA–thymidine] system (each at a concentration of 1.25 mmol·L^{-1})

The speciation of inosine complexes is presented in Fig. 5, where the species distribution of the complexes is plotted as a function of pH. The species 111 is formed in the acidic pH region and it corresponds to the N_7 coordinated complex, while N_1 nitrogen is in the protonated form. The pK_a of the protonated form ($\log_{10} \beta_{111} - \log_{10} \beta_{110}$) amounts to 7.03. In the case of IMP, the protonated species formed correspond to a N_7 coordinated complex, where the N_1 nitrogen and the phosphate group are protonated. The pK_a values of the protonated species of the IMP complex are 6.01 ($\log_{10} \beta_{112} - \log_{10} \beta_{111}$) and 4.84 ($\log_{10} \beta_{111} - \log_{10} \beta_{110}$). The former p*K*a value for corresponds to N_1H group and the second p*K*a value to the-PO_2(OH). The N_1H groups were acidified upon complex formation by 4.37 (9.21 to 4.84) p*K*a units. Acidification of the N_1H group upon complex formation is consistent with previous reports for IMP and AMP complexes [19]. The complexes formed with IMP are more stable than those of pyrimidines.

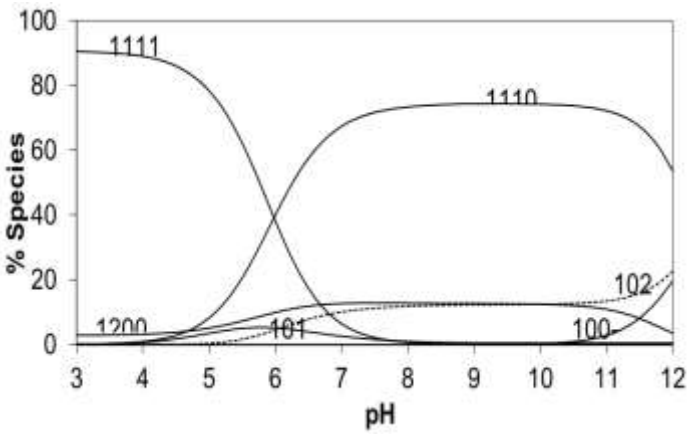

Figure 5. Concentration distribution of various species as a function of pH in the [Cu–PVA–inosine] system (each at a concentration of 1.25 mmol·L^{-1})

TABLE 2 Formation Constants of Cu-PVA-DNA Complexes at Acids At 25^{0}C and 0.1 M Ionic Strength

System	p	q	r[a]	Log$_{10\beta}$[b]	pK$_a$[c]	S[d]/10^{-8}	log$_{10}$K$^{Cu}_{CuD}$
Cu(PVA)	1	1	0	8.07(0.02)			
	1	2	0	15.93(0.03)			
Uracil	0	1	1	9.28(0.006)	9.28	2.4	5.49
	1	1	0	16.61(0.08)		1.5	
Uridine	0	1	1	9.01(0.040)	9.01	8.7	4.03
	1	1	0	16.50(0.02)			
Thymine	0	1	1	9.58(0.040)	9.58	8.7	5.77
	1	1	0	17.70(0.03)			
Thymidine	0	1	1	9.50(0.01)	9.50	20	4.71
	1	1	0	17.56(0.04)			
Inosine	0	1	1	8.43(0.04)	8.43	1.4	4.50
	1	1	0	16.22(0.02)			
	1	1	1	22.20(0.03)	5.98	5.7	5.98
Inosine-5′-monophosphate	0	1	1	9.21(0.04)	9.21	6.11	3.50
	1	1	0	17.60(0.05)		120	
	1	1	1	23.44(0.02)	5.84		
	1	1	2	29.45(0.03)	6.01		
Adenosine-5′-monophosphate	0	1	1	7.17(0.05)	7.17	11	3.13
	0	1	2	10.28(0.06)	3.11		
	1	1	0	18.28(0.03)		20	
	1	1	1	23.48(0.08)	5.20		

Before discussing the results of spectrophotometric measurements, it should be pointed out that the spectrum of aquated copper(II) ion (mixture 1) consists of a broad, weak band (ε = 62*)* with a maximum wave length at 817 nm, attributed to the $2T_2g \leftarrow 2Eg$ transition [21, 22]. The spectral bands of the binary and ternary copper(II) complexes, shown in Fig. 7, 8, are quite different from that of the aquated copper(II) ion, both as regards the position of the maximum wavelength and their average molar absorptivities. The spectrum of the Cu(PVA) complex (curve 3) shows an absorption maximum at 540 nm (ε = 259*)*. On the other hand the spectra obtained from the ternary complex of copper(II) with PVA and glycine or thymidine as a function of equivalents, *a*, of base per pearing at 520 nm (ε = 537) and 540 nm (ε = 537) respectively . This may be taken as evidence, supporting the potentiometric measurement, for induced ionization of the amide

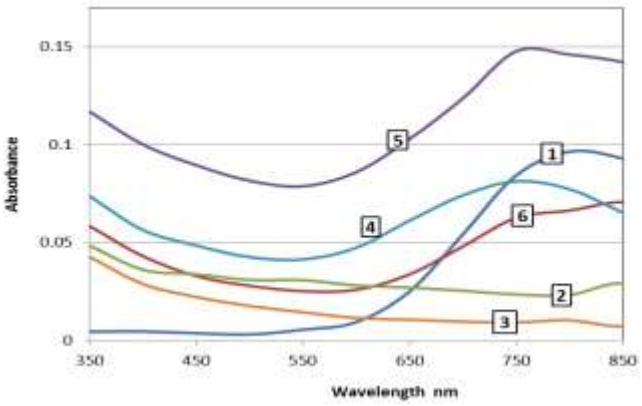

Figure 7. Visible spectra of [Cu-PVA-glycine]: Curves 1.Cu(II) 2.PVA 3.glycine 4.Cu-PVA 5.Cu-glycine 6.Cu-PVA-glycine

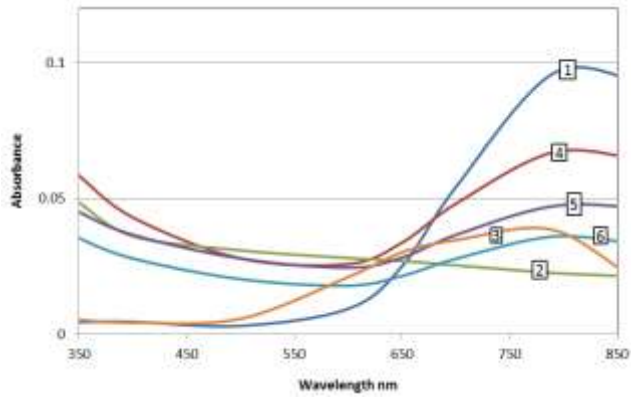

Figure 8. Visible spectra of [Cu-PVA-thymidine]: Curves 1.Cu(II) 2.PVA 3. thymidine 4.Cu-PVA 5.Cu- thymidine 6.Cu-PVA- thymidine

C. *Antimicrobial Activity*

All of the tested compounds show a remarkable biological activity against Gram-positive (G+) and Gram-negative (G−*)* bacteria and also fungi. The data are listed in Table 6. The biological activity of the metal complexes is governed by the following factors [23]: (i) the chelate effect of the ligands, (ii) the nature of the donor atoms, (iii) the total charge on the complex ion, (iv) the nature of the metal ion, (v) the nature of the counter ions that neutralize the complex, and (vi) the geometrical structure of the complex [24]. Furthermore, the increase in biological activity of the metal chelates may be due to the effect of the metal ion on the normal cell process. A possible mode of toxicity may be considered in the light of Tweedy's chelation theory [25]. Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with the donor group and possible π-electron delocalization within the whole chelate ring system that is formed during coordination. Such chelation could enhance the lipophilic character of the central metal atom and hence increase the hydrophobic character and liposolubility of the complex favoring its permeation through the lipid layers of the cell membrane. This enhances the rate of uptake/entrance and thus the antimicrobial activity of the tested compounds. Accordingly, the antimicrobial activity of the complexes present in this investigation can be referred to the increase of their lipophilic character, which in turn deactivates enzymes responsible for respiration processes and probably other cellular enzymes, which play vital roles in various metabolic pathways of the tested micro-organisms. On comparing the biological activity. The results of the antibacterial and antifungal activity of the Cu-PVA-glycine and Cu-PVA-thymidine as representative complexes are recorded in Table 3. The activity of the complexes has been compared with the activity of a common standard tetracycline (antibacterial agent) and amphotricine B (antifungal agent), the following results are obtained:

- Using Escherichia coli (G−) and Staphylococcus aureus (G+): the biological activity of the PVA-Cu-Glycine complex is higher than that of the PVA-Cu-thrymidine complex and slightly lower than that of the tetracycline standard.
- Using Aspergillus flavus fungus: the antifungal activity of the Cu-PVA and the glycine complex are higher than that of the Cu-PVA-thymidine but lower than that standard antifungal agent Amphotericin B
- Using Candida albicans fungus: the biological activity of the Cu-PVA with glycine or thymidine complexes is found to be have lower values than that of the standard Candida albicans.

The tested complexes were more active against Gram +ve than Gram -ve bacteria; it may be concluded that the antibacterial activity of the compounds is related to cell wall structure of the bacteria. The differences in cell wall structure can produce differences in antibacterial susceptibility and some antibiotics can kill only Gram +ve bacteria and are ineffective against Gram -ve pathogens [26, 27].

Conclusions

The formation equilibria of Cu(II) complexes involving PVA and some ligands of biological significance were investigated. In combination with stability constants data of such [Cu(PVA)] complexes with amino acids and DNA constituents, it will be possible to calculate the equilibrium distribution of the metal species in biological fluids where all types of ligands are present simultaneously. This would form a clear basis for understanding the mode of action of such metal species under physiological conditions. The amino acids complexes are more stable than those of the DNA constituents. The β-alcoholate group in the side chain of the amino acid threonine have been found to play an essential role in the function of a number of proteolytic enzymes, for example chymotrypsin and subtilisin. Cu(PVA) promotes the ionization of the alcohol group of threonine with pK_a value of 8.6. This indicates that the participation of the OH group in complex formation is not contributing significantly in the physiological pH range. The results of antimicrobial activity show that the tested complexes were more active against Gram-negative than Gram-positive bacteria. The importance of this lies in the fact that such compounds may have a possible antitumor effect since Gram-negative bacteria are considered a quantitative microbiological method for testing beneficial and important drugs in both clinical and experimental tumor chemotherapy [22].

TABLE 3 The Antibacterial and Antifungal Activity of the Synthesized Metal Complexes

Compounds	Inhibition zone diameter (mm/mg sample)							
	Bacteria				*Fungi*			
	Gram-negative Escherichiacoli (G−)	*A.I %*	*Gram-Positive Staphylococcus aureus (G+)*	*A.I %*	*Aspergillus flavus*	*A.I %*	*Candida Albicans*	*A.I %*
Cu-PVA-glycine	16	73	17(R)	81	11	69	12	67
Cu-PVA-thymidine	14 (R)	64	15(R)	71	10 (R)	63	8	44
Tetracycline Antibacterial agent	22	100	21	100				
Amphotericine B Antifungunal agent					16	100	18	100

a. R: Repellent (not complete inhibition)
b. A.I : Activity index

References

[1] Prichard, G.J. Poly(vinyl alcohol): Basis principles and uses. Gordon and Breach, New York(1970).

[2] Brunelli, D.D., Barboza, V.C., Joekes, I. and Atvars, T.D.ZMapping phases of poly(vinyl alcohol) and poly(vinyl acetate) blends by Ft-Ir microspectroscopy and optical fluorescence microscopy. *Journal of Applied Po- lymer Science*, **69**, 645-655. (1998).

[3] Garrel, D.R., Gaudreau, P., Zhanf, L.M., Reeves, I. and Brazeau, P. Chronic administration of growth hor- mone-releasing factor increases wound strength and col-lagen maturation in granulation tissue. *The Journal of surgical research*, **51**, 297-302(1991).

[4] Kim, J.H., Kim, J.Y., Lee, Y.M. and Kim, K.Y. Properties and swelling characteristics of cross-linked poly(vinyl alcohol)/chitosan blend membrane. *Journal of Applied Polymer Science*, **45**, 1711-1717(1992).

[5] Saxena, A.K., Marler, J., Benvenuto, M., Willital, G.H. and Vacanti, J.P. Skeletal muscle tissue engineer-ing using isolated myoblasts on synthetic biodegradable polymers: Preliminary studies. *Tissue Engineering*, **5**, 525-532(1999).

[6] Peppas, N.P. and Merril, E.W. Development of semicrystalline poly(vinyl alcohol) hydrogels for bio-medical application. *Journal of Biomedical Materials Re- search*, **11**, 423-434(1977).

[7] Welcher, F.J.: The Analytical Uses of Ethylenediamine Tetraacetic Acid. Van Nostand, Princeton (1965)

[8] Stark, J.G., Wallace, H.G. (eds.): Chemistry Data Book, p. 75. Murray, London (1975)

[9] Mohamed, G.G., Soliman,M.H.: Synthesis, spectroscopic and thermal characterization of sulpiride complexes of iron, manganese, copper, cobalt, nickel, and zinc salts. Antibacterial and antifungal activity.Spectochim. Acta A 341–347 (2010).

[10] Vogel, A.E.: Text Book of Quantitative Chemical Analysis, 5th edn., p. 555. Longman, Harlow (1989).Chap. 15

[11] Stark, J.G., Wallace, H.G. (eds.): Chemistry Data Book, p. 75. Murray, London (1975)

[12] Gans, P., Sabatini, A., Vacca, A.: An improved computer program for the computation of formation constants from potentiometric data. J. Inorg. Chim. Acta **18**, 237–239 (1976)

[13] Pettit, L.: Personal Communication. University of Leeds, Leeds (1993)

[14] Shehata, M.R., Shoukry, M.M., Nasr, F.M., Van Eldik, R.: Complex-formation reactions of dicholoro(Smethyl-L-cysteine)palladium(II) with bio-relevant ligands. Labilization induced by S-donor chelates.Dalton Trans. 779–786 (2008)

[15] Azza A. Shoukry and Wafaa M. Hosny, Cent. Eur. J. chem. Eur(10)(1)59-70(2012)

[16] Wafaa M. Hosny and perihan A. Khalaf-Alaa, Int. J. Electrochem. Sci, 7, (2012)

[17] Sigel, H., Martin, R.B.: Coordination properties of the amide bond stability and structure of metal ion complexes of peptides and related ligands. Chem. Rev. **82**, 385–426 (1982)

[18] Grenouillet, P., Martin, R.P., Rossi, A., Ptak, M.: Interactions between copper(II) ions L- threoinine,L-allo-threonine and L-serine in aqueous solutions. Biochim. Biophys. Acta **322**, 185– 194 (1973)

[19] Siegel, H., Massoud, S.S., Corfu, N.A.: Comparison of the extent of base back binding in complexes of divalent metal ions with guanine (GMP^{2-}), inosine (IMP^{2-}) and adenosine 5- monophosphate (AMP^{2-}). The crucial role of N-7 basicity in metal ion- nucleic base recognition. J. Am. Chem. Soc.116, 2958–2971 (1994).

[20] Sigel, H.: Ternary Cu^{2+} complexes stability, structure, and reactivity. Angew. Chem., Int. Ed. 14, 394–402 (1975)

[21] Cotton, F.A., Wilkinson, G.: Basic Inorganic Chemistry, pp. 353–379. Wiley, New York (1973). Part 3.

[22] Shoukry, M.M., Saeed, A., Khairy, E.M.: Equilibrium and hydrolysis of α-amino acid esters in ternary complexes of copper(II) involving glycyl-L-tyrosine. Transit. Metal Chem. **14**, 347– 350(1989)

[23] Chohan, Z.H.: Antibacterial and antifungal ferrocene incorporated dithiothione and dithioketone compounds.Appl. Organomet. Chem. **20**, 112–116 (2005).

[24] Russell, A.D.: Densification, Sterilization and Preservation, 4th edn. Lee and Febinger, Philadelphia (1991)

[25] Tweedy, B.G.: Possible mechanism for reduction of elemental sulfur by Monilinia fructicola. Phytopathology 55, 910–914 (1964)

[26] Koch, A.L., 2003. Bacterial wall as target for attack: past, present, and future research. Clin. Microbiol. Rev. 16, 673.

[27] Inoue, T., Yamashita, Y., Nishihara, M., Sugiyama, S., Sonoda, Y., Kumabe, T., Yokoyama, M., Tominaga,T.: Therapeutic efficacy of a polymeric micellar doxorubicin infused by convection- enhanced delivery against intracranial 9L brain tumor models. Neuro-oncology **11**, 151–157 (2009)

About Author :

In paper we report:

- a quantitative study of the acid base equilibrium of PVA.
- the binary complex formation equilibria of PVA with Cu(II).
- the stability constant of mixed ligand complexes involving Cu-PVA and some amino acids and DNA constituents are studied and the concentration distributions of the complexes are evaluated.