

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

[ Wafaa M. Hosny ]

**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 potentiometrically. 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<sub>1</sub>). 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;  $[-CH_2-CHOH-]_n$ ) 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<sub>3</sub> acid, Cu(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O 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:

$$A.I = \frac{Z_{tc}}{Z_s} \times 100 \quad (1)$$

$Z_{tc}$ : 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 titropressor and electrode were calibrated with standard buffer solutions, prepared according to NBS specifications[10] at  $25 \pm 0.1^\circ\text{C}$  and  $I = 0.1 \text{ mol dm}^{-3}$ , potassium hydrogen phthalate (pH 4.008) and a mixture of  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  (pH 6.865). A ( $0.10 \text{ mol dm}^{-3}$ ) standard acid solution was titrated with a standard base ( $0.10 \text{ mol dm}^{-3}$ ) to convert the pH meter reading into hydrogen ion concentration. The pH values was plotted against  $\text{p[H]}$ , where the relation  $\text{pH} - \text{p[H]} = 0.5$  was observed for all the titration data. A  $\text{pK}_w$  value of 13.997[11] was used to calculate the  $[\text{OH}^-]$ . The titrations were performed in a thermostated titration vessel equipped with a magnetic stirring system, under purified  $\text{N}_2$  atmosphere using  $0.05 \text{ M NaOH}$  as titrant. The titrations were performed at a constant ionic strength of  $0.1 \text{ mol. dm}^{-3}$  ( $\text{NaNO}_3$ ). The acid dissociation constants of the ligand were determined by titrating a  $40 \text{ ml}$  of ligand solution ( $1.25 \times 10^{-3} \text{ mol dm}^{-3}$ ). The formation constants of the complexes were determined by titrating  $40 \text{ mL}$  of the solution containing metal ion ( $1.25 \times 10^{-4} \text{ mol dm}^{-3}$ ) and ligand ( $1.25 \times 10^{-3} \text{ mol dm}^{-3}$ ). The stability constant values were calculated by using the computer program MINQUAD-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 MINQUAD 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 ( $40 \text{ cm}^3$ ) solution ( $1.25 \times 10^{-3} \text{ mol. dm}^{-3}$ ) of constant ionic strength  $0.1 \text{ mol. dm}^{-3}$  (adjusted with  $\text{NaNO}_3$ ). The stability constant of the  $\text{Cu(PVA)}$  complex was determined by titrating  $40 \text{ cm}^3$  of a solution mixture of  $\text{Cu}^{\text{II}}$  ( $1.25 \times 10^{-3} \text{ mol. dm}^{-3}$ ), PVA ligand ( $2.5 \times 10^{-3} \text{ mol. dm}^{-3}$ ) and  $\text{NaNO}_3$  ( $0.1 \text{ mol. dm}^{-3}$ ). The formation constant of the mixed ligand complexes were determined by titrating solution mixtures containing equivalent amounts of  $\text{Cu}^{\text{II}}$  ( $1.25 \times 10^{-3} \text{ cm}^3$ ), 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  $\text{N}_2$  atmosphere using aqueous  $0.05 \text{ mol. dm}^{-3} \text{ NaOH}$  as titrant. The general four component equilibrium can be written as follows (charges are omitted for simplicity)

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

### 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  $10 \text{ cm}^3$  by water, and the ionic strength is kept constant by using  $\text{NaNO}_3$  ( $0.1 \text{ mol. dm}^{-3}$ )

1.  $1.0 \text{ cm}^3 \text{ Cu(II)} (0.01 \text{ mol. dm}^{-3}) + 1.25 \text{ cm}^3 \text{ NaNO}_3 (0.80 \text{ mol. dm}^{-3})$
2.  $1.0 \text{ cm}^3 \text{ glycine or thymidine} (0.01 \text{ mol. dm}^{-3}) + 1.25 \text{ cm}^3 \text{ NaNO}_3 (0.8 \text{ mol. dm}^{-3})$
3.  $1.0 \text{ cm}^3 \text{ PVA} (0.01 \text{ mol. dm}^{-3}) + 1.25 \text{ cm}^3 \text{ NaNO}_3 (0.8 \text{ mol. dm}^{-3})$
4.  $1.0 \text{ cm}^3 \text{ Cu(II)} (0.01 \text{ mol. dm}^{-3}) + 1.0 \text{ cm}^3 \text{ PVA} (0.01 \text{ mol. dm}^{-3}) + 1.25 \text{ cm}^3 \text{ NaNO}_3 (0.8 \text{ mol. dm}^{-3})$
5.  $1.0 \text{ cm}^3 \text{ Cu(II)} (0.01 \text{ mol. dm}^{-3}) + 1.0 \text{ cm}^3 \text{ glycine or thymidine} (0.01 \text{ mol. dm}^{-3}) + 1.25 \text{ cm}^3 \text{ NaNO}_3 (0.8 \text{ mol. dm}^{-3})$
6.  $1.0 \text{ cm}^3 \text{ Cu(II)} (0.01 \text{ mol. dm}^{-3}) + 1.0 \text{ cm}^3 \text{ PVA} (0.01 \text{ mol. dm}^{-3}) + 1.0 \text{ cm}^3 \text{ glycine or thymidine} (0.01 \text{ mol. dm}^{-3}) + 1.25 \text{ cm}^3 \text{ NaNO}_3 (0.8 \text{ mol. dm}^{-3})$

## 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 ( $\text{pK}_a = 10.89$  at  $25^\circ\text{C}$ ), yielding the anionic species  $\text{PVA}^-$ , which starts to form above  $\text{pH} = 4$  reaching its maximum concentration of  $\approx 90$  at  $\text{pH}$ , the stability of binary complex of  $\text{Cu(II)}$  and different amino acids were studied applying potentiometric measurements. The stability constants of binary complexes are allotted in Table 1, the concentration distribution of  $\text{Cu- glycine}$  and  $\text{Cu-Threonine}$  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  $\text{Cu(II)-PVA}$  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  $\text{Cu-PVA-glycine}$  system and  $\text{Cu-PVA-inosine}$  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:  $\text{Cu(PVA)}^+$ ,  $\text{Cu(PVA)}_2$ ,  $\text{Cu(L)}$ ,  $\text{Cu(L)}_2$ ,  $\text{Cu(PVA)(L)}$ ,  $\text{Cu(PVA)(L-1)}$  and  $\text{Cu(PVA)(LH)}$ . Ornithine ( $\text{H}_2\text{L}$ ) is an  $\alpha$ -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  $\text{Cu(PVA)}^+$  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  $\text{pH} \approx 6.5$ . Species with concentrations less than 5% were neglected in the concentration distribution plot for clarity. Threonine has an extra binding center on the  $\beta$ -alcohol-group. This group was reported to participate in complex formation [17]. The  $\text{p}K_a$  value of the  $\beta$ -alcoholato-group incorporated in the  $\text{Cu(II)}$  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  $\text{Cu}$ -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  $\text{Cu(PVA)}$ , 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

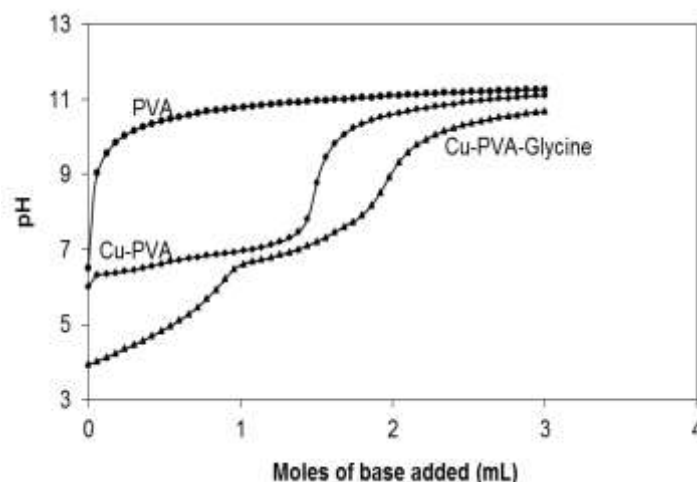


Figure 1. Potentiometric titration curve in the  $[\text{Cu-PVA}]$ -glycine system

TABLE 1 Formation Constants of the Binary and Ternary Complexes in the  $\text{Cu}^{\text{II}}$  PVA Amino Acids at  $25^\circ\text{C}$  and  $0.1 \text{ M}$  Ionic Strength

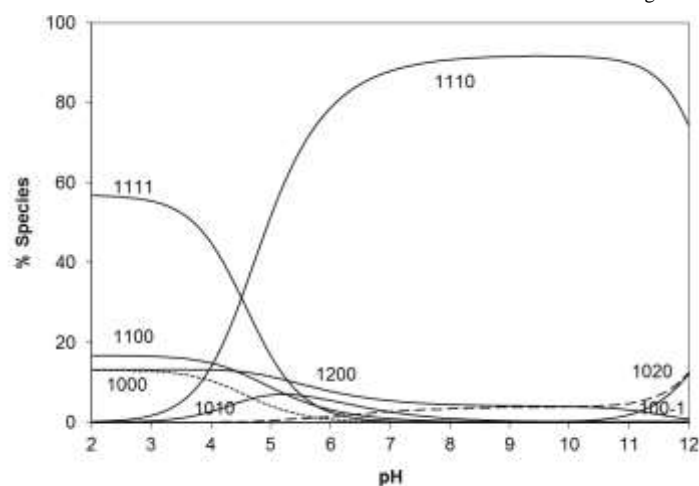


Figure 2. Concentration distribution of various species as a function of pH in the  $[\text{Cu-PVA-glycine}]$  system (each at a concentration of  $1.25 \text{ mmol}\cdot\text{L}^{-1}$ )

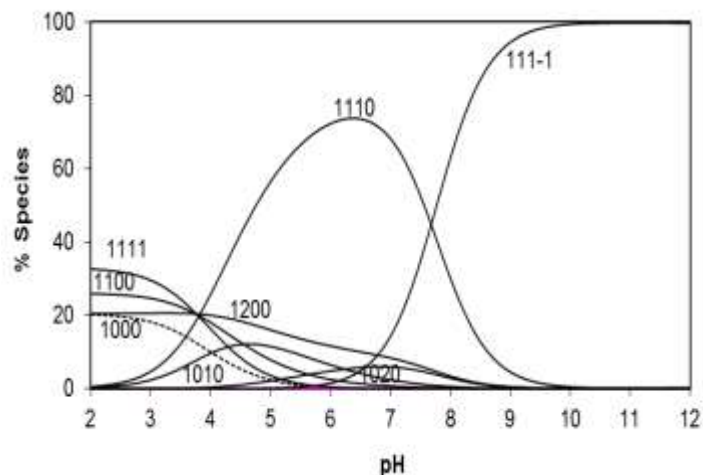


Figure 3. Concentration distribution of various species as a function of pH in the  $[\text{Cu-PVA-threonine}]$  system (each at a concentration of  $1.25 \text{ mmol}\cdot\text{L}^{-1}$ )

Cont. TABLE 1 Formation Constants of the Binary and Ternary Complexes in the Cu<sup>II</sup> PVA Amino Acids at 25°C and 0.1 M Ionic Strength

System	l	p	q	r <sup>a</sup>	Log <sub>10</sub> β <sup>b</sup>	pK <sub>a</sub> <sup>c</sup>	Sd/10 <sup>-8</sup>
[Cu(H <sub>2</sub> O) <sub>4</sub> ] <sup>2+</sup>	1	0	0	-1	-6.44(0.07)		
	1	0	0	-2	12.99(0.02)		9.6x10 <sup>-7</sup>
Cu-PVA	1	1	0	0	8.07(0.01)		1.7x10 <sup>-8</sup>
	1	2	0	0	15.93(0.03)		
Glycine	0	0	1	1	9.60(0.01)	9.60	1.6x10 <sup>-7</sup>
	0	0	1	2	11.93(0.02)	2.33	
	1	0	1	0	8.26(0.07)		3.0x10 <sup>-7</sup>
	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 <sup>-8</sup>
	1	1	1	1	21.32(0.01)	4.51	
Alanine	0	0	1	1	9.69(0.01)	9.69	9.3x10 <sup>-8</sup>
	0	0	1	2	11.89(0.02)	2.20	
	1	0	1	0	7.99(0.03)		3.1x10 <sup>-8</sup>
	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 <sup>-7</sup>
	1	1	1	1	22.00(0.03)	5.55	

<sup>a</sup> l, p, q and r are the stoichiometric coefficients corresponding to Cu<sup>II</sup>, PVA, amino acids and H<sup>+</sup> respectively;  
 the coefficient -1 refers to a proton loss  
<sup>b</sup> log<sub>10</sub> β of Cu-PVA-amino acids. Standard deviation are given in parentheses  
<sup>c</sup> The pK<sub>a</sub> of the ligands and the protonated species  
<sup>d</sup> Sum of squares of residuals

System	l	p	q	r <sup>a</sup>	Log <sub>10</sub> β <sup>b</sup>	pK <sub>a</sub> <sup>c</sup>	Sd/10 <sup>-8</sup>
B-phenyl-alanine	0	0	1	1	9.12(0.01)	9.12	2.0x10 <sup>-8</sup>
	0	0	1	2	11.01(0.03)	1.89	
	1	0	1	0	7.53(0.04)		4.3x10 <sup>-8</sup>
	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 <sup>-7</sup>
Proline	1	1	1	1	20.89(0.03)	4.17	
	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)		
Serine	1	1	1	0	17.40(0.03)		
	1	1	1	1	22.18(0.02)	4.78	
	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(0.02)		
Lysine	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)		
	0	0	1	1	10.44(0.02)	10.44	
	0	0	1	2	19.66(0.02)	9.22	
Methionine	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	
	0	0	1	1	9.13(0.04)	9.13	1.6x10 <sup>-7</sup>
	0	0	1	2	11.10(0.02)	1.97	
	1	0	1	0	8.02(0.04)		
	1	0	2	0	14.79(0.09)		1.6x10 <sup>-7</sup>
	1	0	1	-1	0.78(0.06)		
	1	1	1	1	20.93(0.06)	4.80	

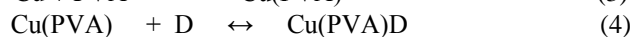


Cont. TABLE 1 Formation Constants of the Binary and Ternary Complexes in the Cu<sup>II</sup> PVA Amino Acids at 25<sup>o</sup>C and 0.1 M Ionic Strength

System	l	p	q	r <sup>a</sup>	Log <sub>10</sub> β <sup>b</sup>	pK <sub>a</sub> <sup>c</sup>	Sd/10 <sup>-8</sup>
Imidazole	0	0	1	1	7.04(0.01)	7.04	1.6x10 <sup>-7</sup>
	1	0	1	0	4.20(0.01)		
	1	0	2	0	7.62(0.04)		1.6x10 <sup>-7</sup>
	1	0	3	0	10.35(0.05)		
	1	1	1	0	16.25(0.08)		1.6x10 <sup>-7</sup>
	1	1	1	1	25.15(0.03)	8.90	
Threonine	0	0	1	1	9.11(0.01)	9.11	7.0x10 <sup>-8</sup>
	0	0	1	2	11.32(0.02)	2.21	
	1	0	1	0	7.97(0.03)		3.1x10 <sup>-8</sup>
	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 <sup>-7</sup>
	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 <sup>-7</sup>
	0	0	1	2	15.81(0.02)	6.28	
	1	0	1	0	11.48(0.01)		1.6x10 <sup>-7</sup>
	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 <sup>-7</sup>
	1	1	1	1	22.36(0.05)	4.83	
Histamine	0	0	1	1	9.88(0.01)	9.88	1.6x10 <sup>-7</sup>
	0	0	1	2	15.97(0.01)	6.09	
	1	0	1	0	10.20(0.01)		1.6x10 <sup>-7</sup>
	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 <sup>-7</sup>
	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<sup>II</sup>-PVA complex is first formed due to its greater stability compared to the Cu<sup>II</sup>-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<sup>II</sup>-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)



The pyrimidinic species (uracil, uridine thymine and thymidine) have only basic nitrogen donor atoms (N<sub>3</sub>-C<sub>4</sub>O 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 basicity of the N<sub>3</sub> of thymidine, resulting from the inductive effect of the extra electron-donating methyl group. As a result of the high pK<sub>a</sub> values of pyrimidines (pK<sub>a</sub> > 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<sub>1</sub> and N<sub>7</sub> 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<sup>3-</sup> ion.

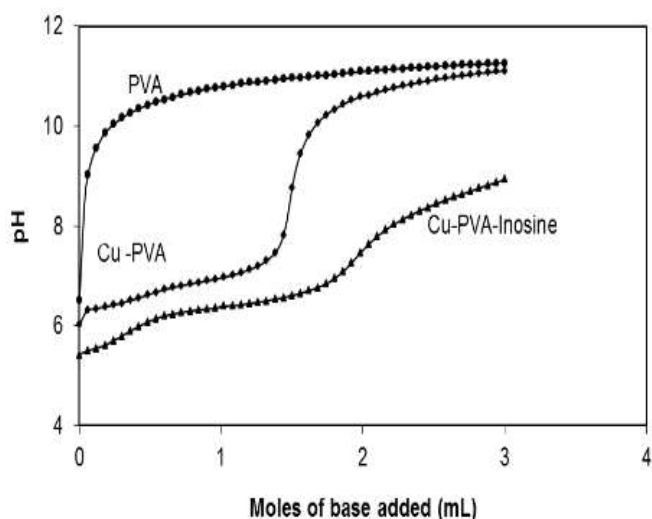


Figure 4. Potentiometric titration curve in the [Cu-PVA]-inosine system

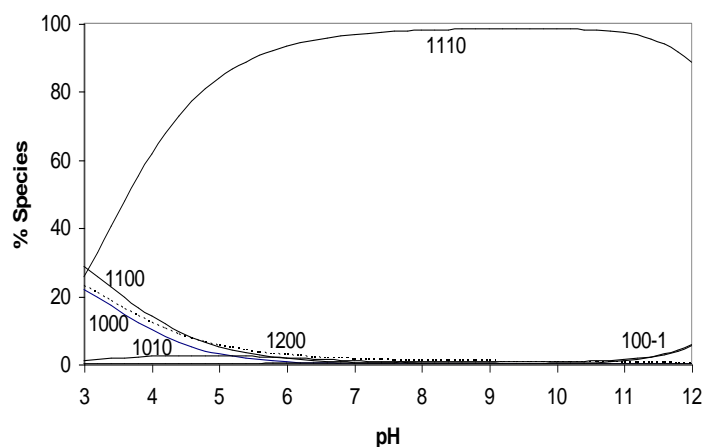


Figure 6. Concentration distribution of various species as a function of pH in the [Cu-PVA-thymidine] system (each at a concentration of 1.25 mmol·L<sup>-1</sup>)

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<sub>7</sub> coordinated complex, while N<sub>1</sub> nitrogen is in the protonated form. The pK<sub>a</sub> of the protonated form (log<sub>10</sub> β<sub>111</sub> - log<sub>10</sub> β<sub>110</sub>) amounts to 7.03. In the case of IMP, the protonated species formed correspond to a N<sub>7</sub> coordinated complex, where the N<sub>1</sub> nitrogen and the phosphate group are protonated. The pK<sub>a</sub> values of the protonated species of the IMP complex are 6.01 (log<sub>10</sub> β<sub>112</sub> - log<sub>10</sub> β<sub>111</sub>) and 4.84 (log<sub>10</sub> β<sub>111</sub> - log<sub>10</sub> β<sub>110</sub>). The former pK<sub>a</sub> value corresponds to N<sub>1</sub>H group and the second pK<sub>a</sub> value to the -PO<sub>2</sub>(OH). The N<sub>1</sub>H groups were acidified upon complex formation by 4.37 (9.21 to 4.84) pK<sub>a</sub> units. Acidification of the N<sub>1</sub>H 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.

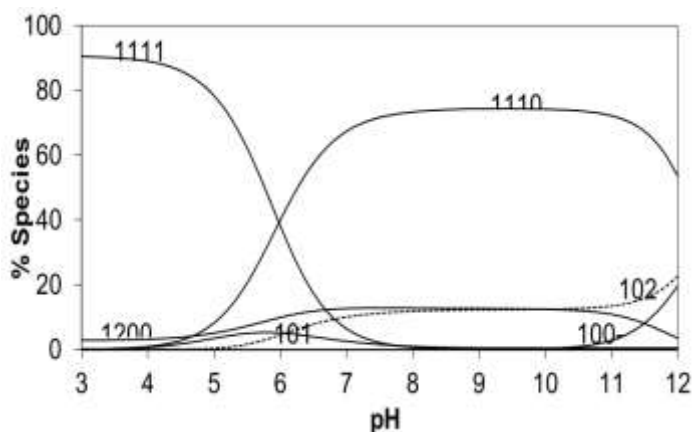


Figure 5. Concentration distribution of various species as a function of pH in the [Cu-PVA-inosine] system (each at a concentration of 1.25 mmol·L<sup>-1</sup>)

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

System	p	q	r <sup>a</sup>	Log <sub>10</sub> <sup>b</sup>	pK <sub>a</sub> <sup>c</sup>	S <sup>d</sup> /10 <sup>-8</sup>	log <sub>10</sub> K <sub>Cu</sub> <sup>Cu</sup> <sub>D</sub>
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		
Adenosine-5'-monophosphate	1	1	2	29.45(0.03)	6.01		
	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 ( $\epsilon = 62$ ) with a maximum wave length at 817 nm, attributed to the  $2T_{2g} \leftarrow 2E_g$  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 ( $\epsilon = 259$ ). 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 peering at 520 nm ( $\epsilon = 537$ ) and 540 nm ( $\epsilon = 537$ ) respectively. This may be taken as evidence, supporting the potentiometric measurement, for induced ionization of the amide

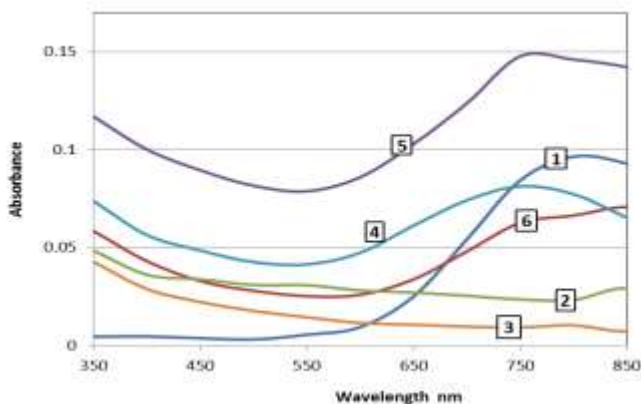


Figure 7. Visible spectra of [Cu-PVA-glycine]: Curves 1.Cu(II) 2.PVA 3.glycine 4.Cu-PVA 5.Cu-glycine 6.Cu-PVA-glycine

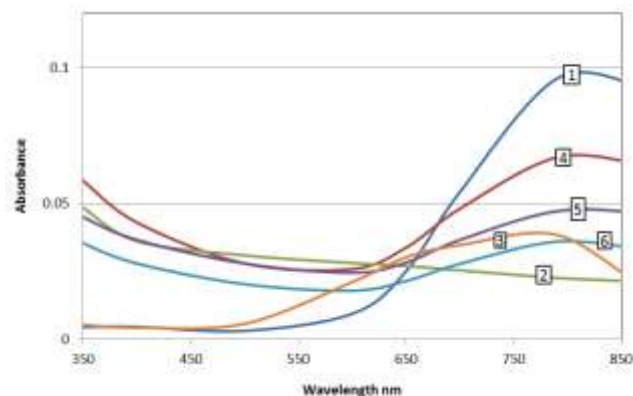


Figure 8. Visible spectra of [Cu-PVA-thymidine]: Curves 1.Cu(II) 2.PVA 3. thymidine 4.Cu-PVA 5.Cu- thymidine 6.Cu-PVA- thymidine

### 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  $\pi$ -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-thymidine 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  $\beta$ -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 <i>Escherichiacoli</i> (G <sup>-</sup> )	A.I %	Gram-Positive <i>Staphylococcus</i> <i>aureus</i> (G <sup>+</sup> )	A.I %	<i>Aspergillus</i> <i>flavus</i>	A.I %	<i>Candida</i> <i>Albicans</i>	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 Antifungal agent					16	100	18	100

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

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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.