Metal Ion Complexes Containing Nucleobases and Some Zwitterionic Buffers

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Potentiometric equilibrium measurements have been performed at (25.0 ± 0.1) °C and ionic strength I = 0.1 mol·dm^{-3} (KNO_3) for the interaction of guanine (2-amino-6-oxypurine), thymine (2,4-dihydroxy-5-methylpyrimidine), adenine (6-aminopurine), uracil (2,4-dioxypyrimidine), hypoxanthine (6-oxypurine), and Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Ca(II), and Mg(II) with the biologically important secondary ligands glycine (aminoethanoic acid), bicine [*N*,*N*-bis(2-hydroxyethyl)glycine], tricine [*N*,*N*,*N*-tris(hydroxymethyl)methylglycine], and ADA [*N*-(2-acetamido)-3-iminodiacetic acid] in a 1:1:1 ratio. The experimental conditions were selected such that self-association of the nucleobases and their complexes was negligibly small; that is, the monomeric normal and protonated complexes were studied. The formation of various 1:1:1 mixed ligand complexes was inferred from the potentiometric titration curves. Initial estimates of the formation constants of the resulting species and the acid dissociation constants of guanine, thymine, adenine, uracil, hypoxanthine, and the secondary ligands glycine, bicine, tricine, and ADA have been refined with the SUPERQUAD computer program. Confirmation of the formation of ternary complexes of the type Cu(II) + NB + Z in solution has been carried out using differential pulse polarography (DPP), square wave voltammetry (SWV), cyclic voltammetry (CV), and UV−visible spectroscopic measurements.

Introduction

The metal complexes of purine and pyrimidine nucleobases play a dominant role in many biochemical systems. The participation of metal ions in biochemical reactions of nucleobases and nucleic acids provided a great interest in determination of structures of metal nucleobase complexes. Heavy-metal toxicity in our environment arises in part from the covalent interactions of heavy-metal ions with nucleic acids. In addition, these heavy metals interfere with metalloregulatory proteins and in so doing disrupt gene expression.

It is important to understand the functioning of the natural metalloregulators of gene expression, and new metal-specific ligands need to be designed, which, like the proteins themselves, capture heavy metals before their damage is done.

Ternary complexes of some metal ions with purine and pyrimidine bases and secondary ligands have been investigated using several techniques.[1−13]

The N-substituted amino acid zwitterionic buffers were prepared by Good[14] and used as buffers in biological research. Bates et al.[15] have found that bicine [*N*,*N*-bis(2-hydroxyethyl)glycine], tricine [*N*,*N*,*N*-tris(hydroxymethyl)-methylglycine], and ADA [*N*-(2-acetamido)-3-iminodiacetic acid] are useful buffers standard in the physiological pH range 6.0−8.5.

Ternary complexes of various metal ions with the N-substituted amino acid zwitterionic buffers have been studied.[16−18]

Artificial chemical DNA nucleases frequently are based on metal−protein conjugates,[19] thereby representing an application of ternary complex formation in molecular biology.

For an improved understanding of the mechanism leading to mixed ligand complexes, the systems M(II) + NB + Z, where NB = guanine, thymine, adenine, uracil, or hypoxanthine, Z = glycine, bicine, tricine, or ADA, and M(II) = Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Ca(II), or Mg(II), have been investigated by potentiometric pH titration. The stability constants of the normal and protonated mixed ligand complexes formed in solution have been determined. These systems mimic many biological reactions [M(II) + buffer + substrate interactions] and also may be considered as models for protein + M(II) + nucleobase complexes. Metal ions such as Mg(II), Mn(II), or Zn(II) have an important biological role in virtually every stage of gene expression involving DNA replication, transcription, and messenger ribonucleic acid (RNA) translation.[20]

The present investigation is an extension of our earlier work on the metal complexes, which play a dominant role in many biochemical systems.[21−26]

Experimental Section

Materials and Solutions. Reagent grade glycine ($C_2H_5NO_2$), bicine ($C_6H_{13}NO_4$), tricine ($C_6H_{13}NO_5$), and ADA ($C_6H_8N_2O_5Na_2$) were from Sigma Chemical Co. Potentiometric pH titrations were used to determine the molecular weight of glycine, bicine, tricine, and ADA to verify the purity, especially for acidic−basic contaminants. The purity averages 99.5% for the four ligand compounds, with a standard deviation of 0.05%.

Guanine ($C_6H_5N_5O$), thymine ($C_5H_6N_2O_2$), adenine ($C_5H_5N_5$), uracil ($C_4H_4N_2O_2$), and hypoxanthine ($C_5H_4N_4O$) were purchased from Sigma Chemical Co. To avoid hydrolysis prior to the potentiometric measurements, a known mass of the chromatographically pure sample of nucleobase as solid was added to the reaction vessel just prior to performing the titration.

Copper nitrate [$Cu(NO_3)_2{\cdot}6H_2O$], nickel nitrate [$Ni(NO_3)_2{\cdot}6H_2O$], cobalt nitrate [$Co(NO_3)_2{\cdot}6H_2O$], manganese nitrate [$Mn(NO_3)_2{\cdot}6H_2O$], and zinc nitrate [$Zn(NO_3)_2{\cdot}6H_2O$] were from Merck p.a. Calcium nitrate [$Ca(NO_3)_2{\cdot}6H_2O$] and magnesium nitrate [$Mg(NO_3)_2{\cdot}6H_2O$] were from BDH.

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Nitric acid and KOH were from Merck p.a. Stock solutions were prepared using bidistilled, CO_2-free water. The concentration of KOH was determined by titrations with a standard solution of potassium hydrogen phthalate (Merck AG).

HNO_3 solutions were prepared and standardized potentiometrically with tris(hydroxymethyl)aminomethane.

The concentrations of the metal ion stock solutions were determined by titration with ethylenediaminetetraacetic acid disodium salt.[27]

Apparatus. Potentiometric pH measurements were made on the solutions in a double-walled glass vessel at (25.0 ± 0.1) °C with a commercial Fisher combined electrode, and a magnetic stirrer was used. A Fisher Accumet pH/ion meter model 825 MP was used. Purified nitrogen was bubbled through the solutions during titrations.

Procedure. The test solution corresponds to a blank without nucleobases titrated with standard CO_2 -free KOH. The electrodes were calibrated, in both the acidic and alkaline regions, by titrating 0.01 mol·dm^{-3} nitric acid with standard potassium hydroxide under the same experimental conditions. The concentration of free hydrogen ion, C_{H^+}, at each point of the titration is related to the measured $E°$ of the cell by the Nernst equation.

$$E = E° + Q \log C_{H^+} \quad (1)$$

where $E°$ is a constant which includes the standard potential of the glass electrode and Q is the slope of the glass electrode response.

The value of $E°$ for the electrode was determined from a Gran plot derived from a separate titration of nitric acid with a standard KOH solution under the same temperature and medium conditions as those for the test solution titration. The results so obtained were analyzed by the nonlinear least squares computer program ESAB2 M[28] to refine $E°$ and the autoprotolysis constant of water, K_w.

During these calculations K_w was refined until the best value for Q was obtained. The results obtained indicated the reversible Nernstian response of the glass electrode used.

The solutions titrated can be presented according to the following scheme: HNO_3 (4×10^{-3} mol·dm^{-3}) + nucleobase (1×10^{-3} mol·dm^{-3}) (a); HNO_3 (4×10^{-3} mol·dm^{-3}) + nucleobase (1×10^{-3} mol·dm^{-3}) + M(II) (1×10^{-3} mol·dm^{-3}) (b); HNO_3 (4×10^{-3} mol·dm^{-3}) + zwitterionic buffer ligand (1×10^{-3} mol·dm^{-3}) (c); HNO_3 (4×10^{-3} mol·dm^{-3}) + zwitterionic buffer ligand (1×10^{-3} mol·dm^{-3}) + M(II) (1×10^{-3} mol·dm^{-3}) (d); HNO_3 (4×10^{-3} mol·dm^{-3}) + nucleobase (1×10^{-3} mol·dm^{-3}) + zwitterionic buffer (1×10^{-3} mol·dm^{-3}) + M(II) (1×10^{-3} mol·dm^{-3}) (e).

A constant ionic strength was obtained with 0.1 mol·dm^{-3} KNO_3, and the total volume was kept at 10.0 cm^3. At least four titrations were performed for each system.

For both ligand protonation and metal complex formation equilibria, data were collected over the largest possible pH interval, although a number of experimental points were frequently discarded for the final stability constant calculations, especially within the range where the complexation observed was insignificant. Typically about a large data set was collected for each system.

Initial estimates of the formation constants of the normal and protonated ternary complexes and the stability constants of the binary 1:1 complexes have been refined using the SUPERQUAD computer program.[29]

During this refinement the stepwise stability constant,

$$K^{M(II)Z}_{M(II)(Z)(NB)} = \frac{[M_p(NB)_q(Z)_r]}{[M_pZ_r][NB]^q}$$

which refers to the addition of NB to the binary complex M_pZ_r, to form the ternary complex $M_p(NB)_q(Z)_r$.

The overall complexation reaction involving protonation is

$$pM + qNB + rZ + sH \rightleftharpoons M_p(NB)_q(Z)_r(H)_s \quad (2)$$

$$\beta_{pqrs} = \frac{[M_p(NB)_q(Z)_r(H)_s]}{[M]^p[NB]^q[Z]^r[H]^s} \quad (3)$$

in which NB = nucleobase (guanine, thymine, adenine, uracil, or hypoxanthine), Z= zwitterionic buffer ligands (bicine, tricine, ADA) or glycine, and M= Cu(II), Co(II), Ni(II), Mn(II), Zn(II), Ca(II), or Mg(II). p, q, r, and s are the moles of M, NB, Z, and H in M_p, $(NB)_q$, $(Z)_r$, and $(H)_s$, respectively.

Electrochemical Measurements. Cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry measurements are collected using an EG and G Princeton applied research, potentiostat/galvanostat model 263 with a single compartment voltammetric cell equipped with a glassy carbon (GC) working electrode (area = 0.1963 cm^2) embedded in a resin, a Pt-wire counter electrode, and an Ag/AgCl reference electrode. In a typical experiment, a sample volume of 25 cm^3 containing the free metal ion 1×10^{-3} mol·dm^{-3} Cu(II) (a), 1×10^{-3} mol·dm^{-3} Cu(II) + 1×10^{-3} mol·dm^{-3} guanine (b), 1×10^{-3} mol·dm^{-3} Cu(II) + 1×10^{-3} mol·dm^{-3} zwitterionic buffer (Z = glycine, bicine, tricine, or ADA) (c), or 1×10^{-3} mol·dm^{-3} Cu(II) + 1×10^{-3} mol·dm^{-3} guanine + 1×10^{-3} mol·dm^{-3} zwitterionic buffer (d) was used. The ionic strength of the studied solutions was adjusted at 0.1 using a KNO_3 solution.

Cyclic Voltammetry. The solution was purged with nitrogen for 120 s, and then the potential was scanned at the scan rate 100 mV·s^{-1} from (+0.20 to −0.30) V.

Square Wave Voltammetry. The samples were analyzed as in cyclic voltammetry, the pulse height was 25 mV, the SW frequency was f = 20 Hz, and the scan increment was dE = 2.0 mV.

Differential Pulse Voltammetry. The samples were analyzed also as in cyclic voltammetry, but at the scan rate = 36.6 mV·s^{-1}. The pulse height was 25 mV, the pulse width = 50 s, frequency = 20 Hz, and the scan increment was 2.0 mV.

Results and Discussion

The second acid formation constants determined at (25.0 ± 0.1) °C for glycine (pK_{a2} = 9.62 ± 0.02), bicine (pK_{a2} = 8.30 ± 0.02), tricine (pK_{a2} = 8.40 ± 0.02), and ADA (pK_{a2} = 6.70 ± 0.02) were in good agreement with those found in the literature.[14,30,31]

The acid formation constant values for guanine (pK_{a2} = 9.21 ± 0.02), thymine (pK_{a2} =9.79 ± 0.01), uracil (pK_{a2} = 9.55 ± 0.02), hypoxanthine (pK_{a2} = 8.89 ± 0.01), and cytosine (pK_{a2} = 4.72 ± 0.02) and the stability constants of their M(II) complexes were determined from the titration curves, and the results were found to agree well with those reported in the literature.[5,32]

Chart 1 shows the predominant tautomeric structures of the purine and pyrimidine bases used in this study with the current numbering system.

Table 1. Formation Constants for the Binary Cu(II) + Nucleobase (NB) or Zwitterionic Buffer (Z) Ligand Complexes and Those for the Mixed Complexes Cu(II) + NB + Z at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

ligand	$\log K^{Cu(II)}_{Cu(II)(Z)}$ or $\log K^{Cu(II)}_{Cu(II)(NB)}$	$\log K^{Cu(II)(glycine)}_{Cu(II)(glycine)(NB)}$ or $\log \beta^{Cu(II)}_{Cu(II)(glycine)(NB)}$	$\log K^{Cu(II)(bicine)}_{Cu(II)(bicine)(NB)}$ or $\log \beta^{Cu(II)}_{Cu(II)(bicine)(NB)}$	$\log K^{Cu(II)(tricine)}_{Cu(II)(tricine)(NB)}$ or $\log \beta^{Cu(II)}_{Cu(II)(tricine)(NB)}$	$\log K^{Cu(II)(ADA)}_{Cu(II)(ADA)(NB)}$ or $\log \beta^{Cu(II)}_{Cu(II)(ADA)(NB)}$
glycine	8.20 ± 0.02				
bicine	7.23 ± 0.02				
tricine	7.58 ± 0.02				
ADA	8.28 ± 0.02				
guanine		5.94 ± 0.03	5.99[b] ± 0.03	6.01[b] ± 0.03	5.49[b] ± 0.03
	6.66 ± 0.03	14.14 ± 0.03			
thymine		7.14 ± 0.02	6.96 ± 0.03	6.87 ± 0.02	6.69[b] ± 0.03
	6.78 ± 0.02	15.34 ± 0.02	14.19 ± 0.03	14.45 ± 0.02	
adenine		8.22[b] ± 0.02	7.78[b] ± 0.03	7.85[b] ± 0.04	8.02[b] ± 0.02
	2.73 ± 0.03				
uracil		6.33 ± 0.02	6.09 ± 0.02	6.05 ± 0.04	4.07 ± 0.02
	6.30 ± 0.05	14.53 ± 0.02	13.32 ± 0.02	13.63 ± 0.04	12.35 ± 0.02
hypoxanthine		6.87 ± 0.03	6.24 ± 0.03	6.26 ± 0.04	4.73[b] ± 0.03
	3.52 ± 0.02	15.07 ± 0.03	13.47 ± 0.03	13.84 ± 0.04	

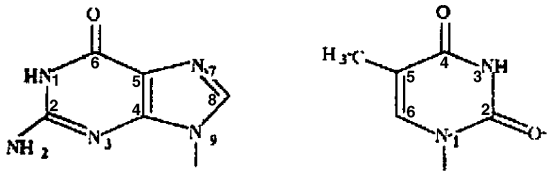
[a] Log $\beta^{Cu(II)}_{Cu(II)(Z)(NB)} = \log K^{Cu(II)}_{Cu(II)(Z)(NB)} + \log K^{Cu(II)}_{Cu(II)(Z)}$; ± uncertainties refer to 3 times the standard deviation (3s). [b] Log formation constant of protonated ternary complex: $\log K^{Cu(II)}_{Cu(II)(Z)(HNB)}$.

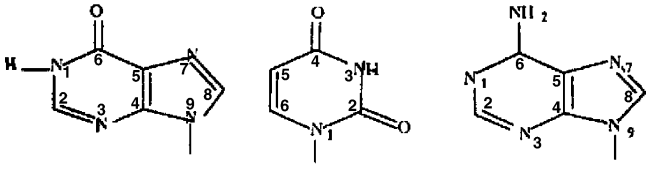
Table 2. Formation Constants for the Binary Ni(II) + Nucleobase (NB) or Zwitterionic Buffer (Z) Ligand Complexes and Those for the Mixed Complexes Ni(II) + NB + Z at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

ligand	$\log K^{Ni(II)}_{Ni(II)(Z)}$ or $\log K^{Ni(II)}_{Ni(II)(NB)}$	$\log K^{Ni(II)(glycine)}_{Ni(II)(glycine)(NB)}$ or $\log \beta^{Ni(II)}_{Ni(II)(glycine)(NB)}$	$\log K^{Ni(II)(bicine)}_{Ni(II)(bicine)(NB)}$ or $\log \beta^{Ni(II)}_{Ni(II)(bicine)(NB)}$	$\log K^{Ni(II)(tricine)}_{Ni(II)(tricine)(NB)}$ or $\log \beta^{Ni(II)}_{Ni(II)(tricine)(NB)}$	$\log K^{Ni(II)(ADA)}_{Ni(II)(ADA)(NB)}$ or $\log \beta^{Ni(II)}_{Ni(II)(ADA)(NB)}$
glycine	5.49 ± 0.02				
bicine	4.69 ± 0.02				
tricine	5.21 ± 0.02				
ADA	6.80 ± 0.02				
guanine		3.99 ± 0.03	5.87 ± 0.03	4.89 ± 0.02	4.84[b] ± 0.03
	4.25 ± 0.02	9.48 ± 0.03	10.56 ± 0.03	10.10 ± 0.02	
thymine		4.70 ± 0.02	4.39 ± 0.02	4.76 ± 0.03	4.15[b] ± 0.03
	4.81 ± 0.05	10.19 ± 0.02	9.08 ± 0.02	9.97 ± 0.03	
adenine		5.72[b] ± 0.02	4.87[b] ± 0.02	5.34[b] ± 0.02	5.41[b] ± 0.03
	4.40 ± 0.00				
uracil		4.18 ± 0.03	4.17 ± 0.02	4.22 ± 0.03	3.57 ± 0.02
	4.24 ± 0.05	9.67 ± 0.03	8.86 ± 0.02	9.43 ± 0.03	10.37 ± 0.02
hypoxanthine		4.31 ± 0.02	4.08 ± 0.03	4.13 ± 0.02	3.60[b] ± 0.02
	2.80 ± 0.02	9.80 ± 0.02	8.77 ± 0.03	9.34 ± 0.02	

[a] Log $\beta^{Ni(II)}_{Ni(II)(Z)(NB)} = \log K^{Ni(II)}_{Ni(II)(Z)(NB)} + \log K^{Ni(II)}_{Ni(II)(Z)}$; ± uncertainties refer to 3 times the standard deviation (3s). [b] Log formation constant of protonated ternary complex: $\log K^{Ni(II)}_{Ni(II)(Z)(HNB)}$.

Chart 1





The proton binding sites of the purine nucleic bases have been established (N3 for cytosine and uracil; protonation of N between the keto groups in thymine and between N1 and N7 for guanine and hypoxanthine). The N1 site in the six-membered ring is at least 100 times more basic than the N7 site in the five-membered ring. While both N1 and N7 in purine bases bind metal ions, they exhibit different features. In the absence of steric hindrance, the N7/N1 binding ratio for the first transition row metal ions Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} is approximately 2.[5]

At the experimental pH values used in the calculation in this work, the interfering effects of hydroxy complexes are negligible [for Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Ca(II), or Mg(II)]. Thus, the secondary ligand combines with the binary 1:1 M(II)−nucleobase complexes [M(II)−guanine, M(II)−thymine, M(II)−adenine, M(II)−uracil, and M(II)−hypoxanthine] in a manner similar to that for its interaction with aquated metal ions in solutions. Thus, the initial estimates of the stability constants of the normal ternary complexes formed in solution have been determined using the Irving and Rossotti formula.[33,34]

Initial estimates of the stability constants of different normal and protonated binary and ternary complexes formed in solution have been refined with the SUPERQUAD computer program.[29]

The quality of the fit during this refinement was judged by the values of the sample standard deviations and the goodness of fit χ^2 (Pearson's Test). At σ_E = 0.1 mV (0.001 pH error) and σ_v = 0.005 mL, the values of S in different sets of titrations were between 1.0 and 1.7 and χ^2 was

Table 3. Formation Constants for the Binary Co(II) + Nucleobase (NB) or Zwitterionic Buffer (Z) Ligand Complexes and Those for the Mixed Complexes Co(II) + NB + Z at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

ligand	$\log K^{Co(II)}_{Co(II)(Z)}$ or $\log K^{Co(II)}_{Co(II)(NB)}$	$\log K^{Co(II)(glycine)}_{Co(II)(glycine)(NB)}$ or $\log \beta^{Co(II)}_{Co(II)(glycine)(NB)}$	$\log K^{Co(II)(bicine)}_{Co(II)(bicine)(NB)}$ or $\log \beta^{Co(II)}_{Co(II)(bicine)(NB)}$	$\log K^{Co(II)(tricine)}_{Co(II)(tricine)(NB)}$ or $\log \beta^{Co(II)}_{Co(II)(tricine)(NB)}$	$\log K^{Co(II)(ADA)}_{Co(II)(ADA)(NB)}$ or $\log \beta^{Co(II)}_{Co(II)(ADA)(NB)}$
glycine	4.60 ± 0.02				
bicine	4.92 ± 0.02				
tricine	5.44 ± 0.03				
ADA	6.34 ± 0.03				
guanine		3.96 ± 0.03	5.20 ± 0.03	4.50 ± 0.03	4.87[b] ± 0.03
	4.16 ± 0.03	8.56 ± 0.03	10.12 ± 0.03	9.94 ± 0.03	
thymine		4.53 ± 0.03	4.89 ± 0.03	4.71 ± 0.02	4.11 ± 0.02
	4.40 ± 0.02	9.13 ± 0.03	10.10 ± 0.03	10.15 ± 0.02	10.45 ± 0.02
adenine		5.12[b] ± 0.04	4.70[b] ± 0.02	5.27[b] ± 0.03	4.74[b] ± 0.02
	1.90 ± 0.00				
uracil		4.15 ± 0.03	3.97 ± 0.02	4.04 ± 0.03	3.37 ± 0.02
	3.86 ± 0.03	8.75 ± 0.03	9.18 ± 0.02	9.48 ± 0.03	9.71 ± 0.02
hypoxanthine		4.06 ± 0.03	3.99 ± 0.02	4.01 ± 0.03	3.55[b] ± 0.02
	2.51 ± 0.02	8.66 ± 0.03	9.20 ± 0.02	9.45 ± 0.03	

[a] Log $\beta^{Co(II)}_{Co(II)(Z)(NB)} = \log K^{Co(II)}_{Co(II)(Z)(NB)} + \log K^{Co(II)}_{Co(II)(Z)}$; ± uncertainties refer to 3 times the standard deviation (3s). [b] Log formation constant of protonated ternary complex: $\log K^{Co(II)}_{Co(II)(Z)(HNB)}$.

Table 4. Formation Constants for the Binary Mn(II) + Nucleobase (NB) or Zwitterionic Buffer (Z) Ligand Complexes and Those for the Mixed Complexes Mn(II) + NB + Z at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

ligand	$\log K^{Mn(II)}_{Mn(II)(Z)}$ or $\log K^{Mn(II)}_{Mn(II)(NB)}$	$\log K^{Mn(II)(glycine)}_{Mn(II)(glycine)(NB)}$ or $\log \beta^{Mn(II)}_{Mn(II)(glycine)(NB)}$	$\log K^{Mn(II)(bicine)}_{Mn(II)(bicine)(NB)}$ or $\log \beta^{Mn(II)}_{Mn(II)(bicine)(NB)}$	$\log K^{Mn(II)(tricine)}_{Mn(II)(tricine)(NB)}$ or $\log \beta^{Mn(II)}_{Mn(II)(tricine)(NB)}$	$\log K^{Mn(II)(ADA)}_{Mn(II)(ADA)(NB)}$ or $\log \beta^{Mn(II)}_{Mn(II)(ADA)(NB)}$
glycine	3.00 ± 0.02				
bicine					
tricine					
ADA	5.61 ± 0.02				
guanine		3.95 ± 0.03	4.11 ± 0.03	4.44 ± 0.03	3.95[b] ± 0.02
	4.21 ± 0.02	6.65 ± 0.03			
thymine		5.39 ± 0.03	4.80 ± 0.03	4.83 ± 0.03	4.18 ± 0.02
	3.72 ± 0.02	8.39 ± 0.03			9.79 ± 0.02
adenine		4.55[b] ± 0.02	4.64[b] ± 0.02	4.52[b] ± 0.03	4.52[b] ± 0.02
uracil		4.01 ± 0.03	3.80 ± 0.02	3.74 ± 0.03	3.25 ± 0.02
	3.45 ± 0.03	7.01 ± 0.03			8.86 ± 0.02
hypoxanthine		3.59 ± 0.03	3.63 ± 0.02	3.43 ± 0.03	3.09[b] ± 0.03
		6.59 ± 0.03			

[a] Log $\beta^{Mn(II)}_{Mn(II)(Z)(NB)} = \log K^{Mn(II)}_{Mn(II)(Z)(NB)} + \log K^{Mn(II)}_{Mn(II)(Z)}$; ± uncertainties refer to 3 times the standard deviation (3s). [b] Log formation constant of protonated ternary complex: $\log K^{Mn(II)}_{Mn(II)(Z)(HNB)}$.

Table 5. Formation Constants for the Binary Zn(II) + Nucleobase (NB) or Zwitterionic Buffer (Z) Ligand Complexes and Those for the Mixed Complexes Zn(II) + NB + Z at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

ligand	$\log K^{Zn(II)}_{Zn(II)(Z)}$ or $\log K^{Zn(II)}_{Zn(II)(NB)}$	$\log K^{Zn(II)(glycine)}_{Zn(II)(glycine)(NB)}$ or $\log \beta^{Zn(II)}_{Zn(II)(glycine)(NB)}$	$\log K^{Zn(II)(bicine)}_{Zn(II)(bicine)(NB)}$ or $\log \beta^{Zn(II)}_{Zn(II)(bicine)(NB)}$	$\log K^{Zn(II)(tricine)}_{Zn(II)(tricine)(NB)}$ or $\log \beta^{Zn(II)}_{Zn(II)(tricine)(NB)}$	$\log K^{Zn(II)(ADA)}_{Zn(II)(ADA)(NB)}$ or $\log \beta^{Zn(II)}_{Zn(II)(ADA)(NB)}$
glycine	5.30 ± 0.02				
bicine	5.29 ± 0.02				
tricine	6.23 ± 0.02				
ADA	6.45 ± 0.02				
guanine		4.33± 0.03	5.15 ± 0.02	4.76 ± 0.02	4.05[b] ± 0.03
	6.21 ± 0.02	9.63 ± 0.03	10.44 ± 0.02	10.99 ± 0.02	
thymine		5.38 ± 0.03	5.26 ± 0.04	5.57 ± 0.04	4.58[b] ± 0.02
	5.50 ± 0.03	10.68 ± 0.03	10.55 ± 0.04	11.80 ± 0.04	
adenine		6.21[b] ± 0.04	5.79[b] ± 0.04	5.89[b] ± 0.03	4.97[b] ± 0.02
	2.20 ± 0.02				
uracil		4.79 ± 0.03	4.13 ± 0.03	4.34 ± 0.03	3.45 ± 0.02
	5.10 ± 0.03	10.09 ± 0.03	9.60 ± 0.03	10.57 ± 0.03	9.90 ± 0.02
hypoxanthine		4.42 ± 0.03	4.19 ± 0.02	4.26 ± 0.03	3.47 ± 0.02
	3.01 ± 0.01	9.72 ± 0.03	9.48 ± 0.02	10.49 ± 0.03	9.92 ± 0.02

[a] Log $\beta^{Zn(II)}_{Zn(II)(Z)(NB)} = \log K^{Zn(II)}_{Zn(II)(Z)(NB)} + \log K^{Zn(II)}_{Zn(II)(Z)}$; ± uncertainties refer to 3 times the standard deviation (3s). [b] Log formation constant of protonated ternary complex: $\log K^{Zn(II)}_{Zn(II)(Z)(HNB)}$.

between 12.0 and 13.0. The scatter of residuals ($E_{obs} - E_{calc}$) versus pH was reasonably random, without any significant systematic trends, thus indicating a good fit of the experimental data of the expected model systems under our experimental conditions.

Examination of the different formation constant values listed in Tables 1−7 reveals that the order of the overall stability constants of the different ternary complexes in the systems M(II) + NB + glycine in terms of metal ions follows generally the trend Cu(II) > Zn(II) > Ni(II) > Co(II) >

Table 6. Formation Constants for the Binary Ca(II) + Nucleobase (NB) or Zwitterionic Buffer (Z) Ligand Complexes and Those for the Mixed Complexes Ca(II) + NB + Z at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

ligand	$\log K^{Ca(II)}_{Ca(II)(Z)}$ or $\log K^{Ca(II)}_{Ca(II)(NB)}$	$\log K^{Ca(II)(glycine)}_{Ca(II)(glycine)(NB)}$ or $\log \beta^{Ca(II)}_{Ca(II)(glycine)(NB)}$	$\log K^{Ca(II)(bicine)}_{Ca(II)(bicine)(NB)}$ or $\log \beta^{Ca(II)}_{Ca(II)(bicine)(NB)}$	$\log K^{Ca(II)(tricine)}_{Ca(II)(tricine)(NB)}$ or $\log \beta^{Ca(II)}_{Ca(II)(tricine)(NB)}$	$\log K^{Ca(II)(ADA)}_{Ca(II)(ADA)(NB)}$ or $\log \beta^{Ca(II)}_{Ca(II)(ADA)(NB)}$
glycine	2.86 ± 0.02				
bicine	4.13 ± 0.02				
tricine	4.69 ± 0.02				
ADA	3.29 ± 0.02				
guanine		3.44 ± 0.03	3.78 ± 0.03	4.08 ± 0.03	3.82[b] ± 0.02
	3.34 ± 0.02	6.30 ± 0.03	7.91 ± 0.03	8.77 ± 0.03	
thymine		3.75 ± 0.03	4.19 ± 0.03	3.92 ± 0.03	4.03[b] ± 0.02
	3.14 ± 0.02	6.61 ± 0.03	8.32 ± 0.03	8.61 ± 0.03	
adenine		3.99[b] ± 0.02	4.21[b] ± 0.03	4.29[b] ± 0.04	4.66[b] ± 0.02
	2.10 ± 0.02				
uracil		3.71 ± 0.02	3.48 ± 0.02	3.95 ± 0.04	2.77 ± 0.02
	3.38 ± 0.02	6.57 ± 0.02	7.61 ± 0.02	8.64 ± 0.04	6.06 ± 0.02
hypoxanthine		3.31 ± 0.02	3.64 ± 0.02	3.41 ± 0.03	3.28[b] ± 0.02
	2.20 ± 0.02	6.17 ± 0.02	7.77 ± 0.02	8.10 ± 0.03	

[a] Log $\beta^{Ca(II)}_{Ca(II)(Z)(NB)} = \log K^{Ca(II)}_{Ca(II)(Z)(NB)} + \log K^{Ca(II)}_{Ca(II)(Z)}$; ± uncertainties refer to 3 times the standard deviation (3s). [b] Log formation constant of protonated ternary complex: $\log K^{Ca(II)}_{Ca(II)(Z)(HNB)}$.

Table 7. Formation Constants for the Binary Mg(II) + Nucleobase (NB) or Zwitterionic Buffer (Z) Ligand Complexes and Those for the Mixed Complexes Mg(II) + NB + Z at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

ligand	$\log K^{Mg(II)}_{Mg(II)(Z)}$ or $\log K^{Mg(II)}_{Mg(II)(Z)(NB)}$	$\log K^{Mg(II)(glycine)}_{Mg(II)(glycine)(NB)}$ or $\log \beta^{Mg(II)}_{Mg(II)(glycine)(NB)}$	$\log K^{Mg(II)(bicine)}_{Mg(II)(bicine)(NB)}$ or $\log \beta^{Mg(II)}_{Mg(II)(bicine)(NB)}$	$\log K^{Mg(II)(tricine)}_{Mg(II)(tricine)(NB)}$ or $\log \beta^{Mg(II)}_{Mg(II)(tricine)(NB)}$	$\log K^{Mg(II)(ADA)}_{Mg(II)(ADA)(NB)}$ or $\log \beta^{Mg(II)}_{Mg(II)(ADA)(NB)}$
glycine	3.09 ± 0.02				
bicine	4.05 ± 0.02				
tricine	4.22 ± 0.02				
ADA	3.30 ± 0.02				
guanine		3.36 ± 0.02	3.85 ± 0.02	4.20 ± 0.03	4.68[b] ± 0.02
	3.50 ± 0.02	6.45 ± 0.02	7.90 ± 0.02	8.42 ± 0.03	
thymine		3.55 ± 0.02	4.10 ± 0.03	3.88 ± 0.03	3.90[b] ± 0.02
	3.18 ± 0.02	6.64 ± 0.02	8.15 ± 0.03	8.10 ± 0.03	
adenine		3.98[b] ± 0.02	4.24[b] ± 0.03	4.01[b] ± 0.02	4.66[b] ± 0.02
	2.42 ± 0.01				
uracil		3.71 ± 0.02	3.58 ± 0.02	3.48 ± 0.03	3.07 ± 0.02
	3.26 ± 0.02	6.80 ± 0.03	7.63 ± 0.02	7.70 ± 0.03	6.37 ± 0.02
hypoxanthine		3.37 ± 0.02	3.59 ± 0.02	3.46 ± 0.02	3.42[b] ± 0.02
	2.24±0.02	6.46 ± 0.02	7.64 ± 0.02	7.68 ± 0.02	

[a] log $\beta^{Mg(II)}_{Mg(II)(Z)(NB)} = \log K^{Mg(II)}_{Mg(II)(Z)(NB)} + \log K^{Mg(II)}_{Mg(II)(Z)}$; ± uncertainties refer to 3 times the standard deviation (3s). [b] Log formation constant of protonated ternary complex: $\log K^{Mg(II)}_{Mg(II)(Z)(HNB)}$.

Table 8. Δ log K_M[a] Values for the 1:1:1 M(II) + Zwitterionic Buffer (Z) + Nucleobase (NB) Ternary Complexes As Determined by Potentiometric pH Titrations at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} KNO_3

M(II)(Z)(NB)		Δ log K_M						
Z	NB	Cu(II)	Ni(II)	Co(II)	Mn(II)	Zn(II)	Ca(II)	Mg(II)
glycine	guanine	−0.72	−0.26	−0.20	−0.56	−1.88	+0.10	−0.14
	thymine	+0.17	−0.11	−0.18	+1.10	−0.63	+0.57	+0.17
	uracil	−0.54	−0.06	+0.07	+0.24	+0.19	+0.33	+0.35
	hypoxanthine	+3.37	+1.51	+1.56		+1.41	+1.11	+1.11
	adenine	+5.52	+3.32[b]	+3.22[b]		+4.01[b]	+1.89[b]	+1.58[b]
bicine	guanine		+1.62	+1.04	−1.10	−1.06	−0.44	+0.35
	thymine	−0.01	−0.42	+0.18	+0.51	+0.75	+1.01	+0.22
	uracil	−0.78	−0.07	−0.11	+0.03	−0.29	+0.10	+0.22
	hypoxanthine	+2.74	+1.28	+1.49		+1.18	+1.44	+1.35
	adenine	+5.08[b]	+2.47[b]	+2.80[b]		+3.59[b]	+2.11[b]	+1.84
tricine	guanine		+0.64	+0.34		−11.45	+0.74	
	thymine	−0.10	−0.05	0.00		−0.44	+0.74	
	uracil	−0.82	−0.02	−0.04		−0.26	+0.57	−0.29
	hypoxanthine	+2.76	+1.33	+1.51		+1.25	+1.21	
	adenine	+5.15[b]	+2.94[b]	+3.37[b]		+3.96[b]	+2.19[b]	
ADA	guanine			−0.60	−0.11			
	thymine	−2.80	−0.67	−0.71	−0.52	−1.15	−0.61	−0.29
	uracil					+0.46		
	hypoxanthine							
	adenine							

[a] Δ log $K_M = \log K^{M(II)(Z)}_{M(II)(Z)(NB)} - \log K^{M(II)}_{M(II)(NB)}$ [b] Δ log $K_M = \log K^{M(II)(Z)}_{M(II)(Z)(HNB)} - \log K^{M(II)}_{M(II)(HNB)}$

Mn(II) > Mg (II) > Ca(II), Cu(II) > Ni(II) > Zn(II) > Co(II) > Mn(II) > Mg (II) > Ca(II), for the systems M(II) + glycine + guanine, M(II) + glycine + thymine, M(II) + glycine + uracil, and M(II) + glycine + hypoxanthine, respectively.

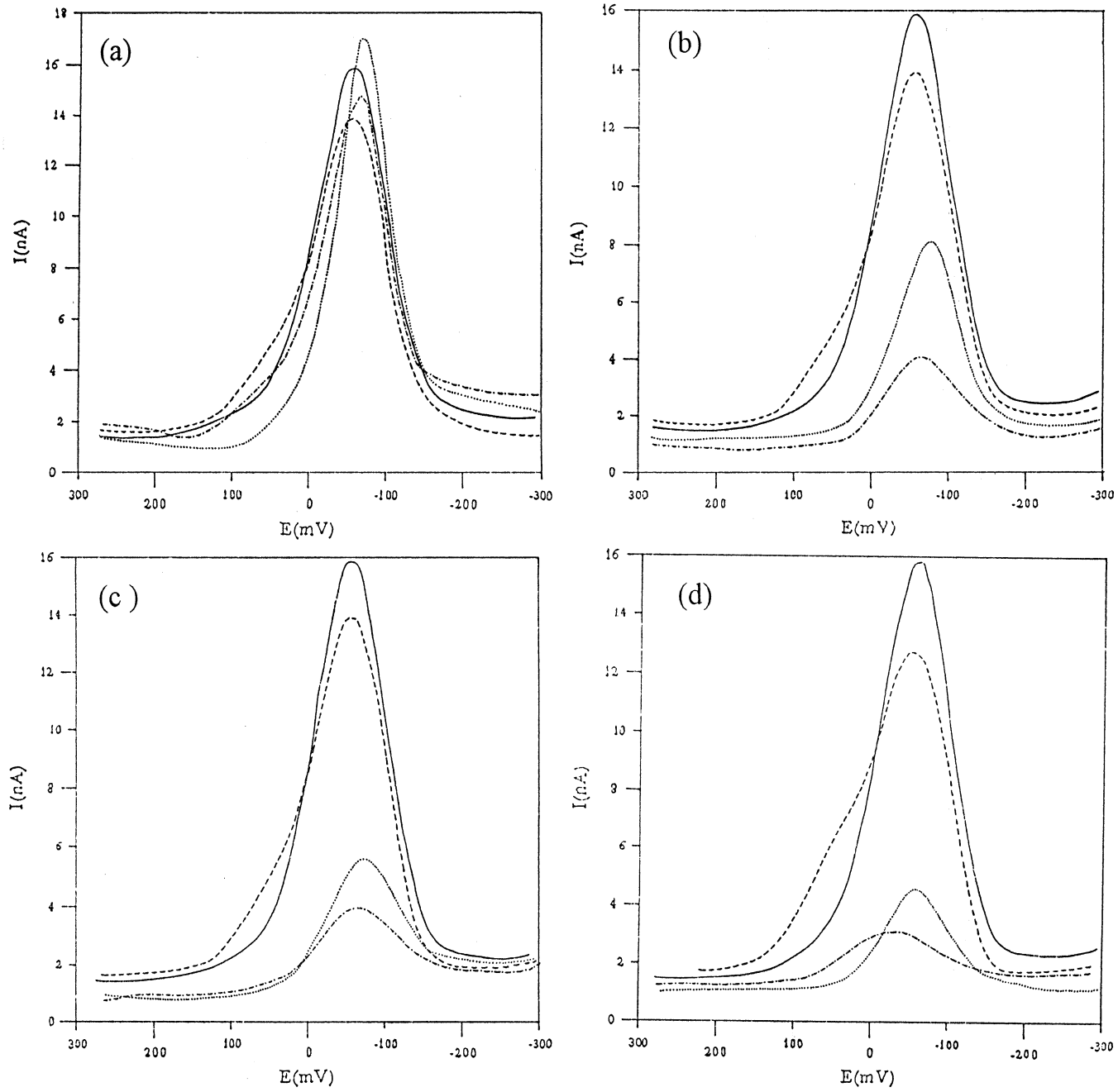


Figure 1. Differential pulse polarograms for the Cu(II) + Z + guanine system at I = 0.1 mol dm^{-3} KNO_3, pH = 6.5, and scan rate = 25 mV/s and at 25 °C: (—) 1 × 10^{-3} mol·dm^{-3} Cu(II); (· · ·) 1 × 10^{-3} mol·dm^{-3} Cu(II) + 1 × 10^{-3} mol·dm^{-3} Z; (- - -) 1 × 10^{-3} mol·dm^{-3} Cu(II) + 1 × 10^{-3} mol·dm^{-3} guanine; (- · -) 1 × 10^{-3} mol·dm^{-3} Cu(II) + 1 × 10^{-3} mol·dm^{-3} Z + 1 × 10^{-3} mol·dm^{-3} guanine. (a) Z = glycine; (b) Z = bicine; (c) Z = tricine; (d) Z = ADA.

For the systems M(II) + bicine + nucleobase the trend was Ni(II) > Zn(II) > Co(II) > Ca(II) > Mg(II), Cu(II) > Zn(II), Co(II) > Ni(II) > Ca(II) > Mg(II), Cu(II) > Zn(II) > Co(II) > Ni(II) > Mg(II) > Ca(II), and Cu(II) > Zn(II) > Co(II) > Ni(II) > Ca(II) > Mg(II) for the systems M(II) + bicine + guanine, M(II) + bicine + thymine, M(II) + bicine + uracil, and M(II) + bicine + hypoxanthine, respectively.

The trend was Zn(II) > Ni(II) > Co(II) > Ca(II) > Mg(II), Cu(II) > Zn(II), Co(II) > Ni(II) > Ca(II) > Mg(II), Cu(II) > Zn(II) > Co(II) > Ni(II) > Ca(II) > Mg(II), and Cu(II) > Zn(II) > Co(II) > Ni(II) > Ca(II) > Mg(II) for the systems M(II) + tricine + guanine, M(II) + tricine + thymine, M(II) + tricine + uracil, and M(II) + tricine + hypoxanthine, respectively. To the author's knowledge no data for the ternary complexes of the secondary ligands glycine, bicine, tricine, or ADA with the nucleobases guanine, thymine, adenine, uracil, or hypoxanthine are available in the literature for comparison. The observed trend for the overall formation constant of the different ternary complexes formed in this study in terms of metal ions may be attributed to the nature of the interaction of the metal ions Cu(II), Ni(II), Co(II), Mn(II), Zn(II), Ca(II), and Mg(II) during their binding to the nucleobases guanine, thymine, uracil, adenine, or hypoxanthine. At neutral pH, the affinity for the metal ion binding of the sites available in the nucleobases in single-stranded nucleic acid decreases along the series[35] N7G > N3C > N7A > N1A > N3A, N3C.

As stated previously,[36] these ions favor mixed oxygen and nitrogen donors. Although N1 is more basic than N7, it was confirmed that macrochelate formation with N1 of the purine moiety is not possible for steric reasons; such an evaluation, which of course reflects the metal ion binding

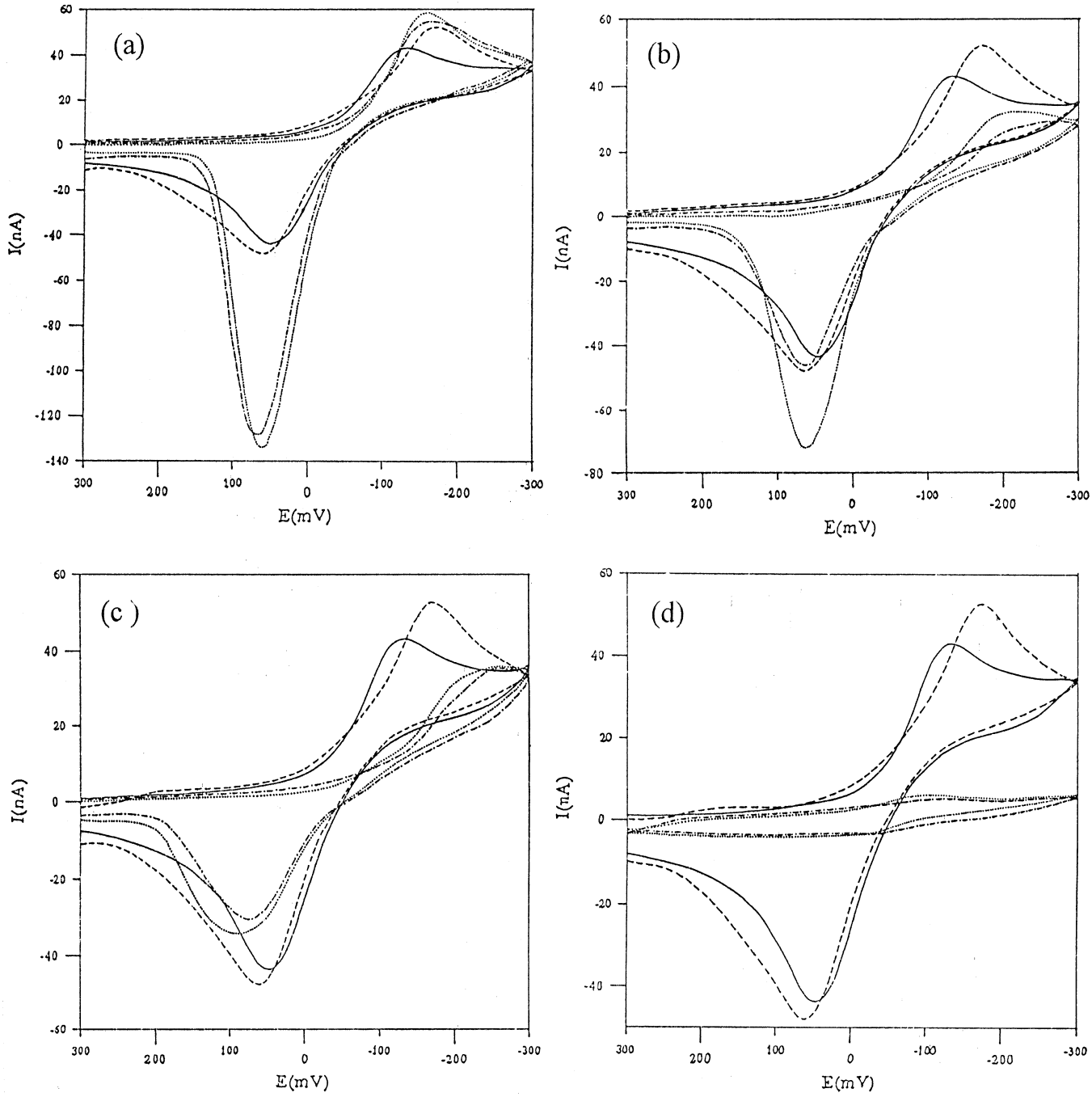


Figure 2. Cyclic voltammograms for the Cu(II) + Z + guanine system at I = 0.1 mol dm^{-3} KNO_3, pH = 6.5, and scan rate = 25 mV/s and at 25 °C: (—) 1 × 10^{-3} mol·dm^{-3} Cu(II); (· · ·) 1 × 10^{-3} mol·dm^{-3} Cu(II) + 1 × 10^{-3} mol·dm^{-3} Z; (- - -) 1 × 10^{-3} mol·dm^{-3} Cu(II) + 1 × 10^{-3} mol·dm^{-3} guanine; (- · -) 1 × 10^{-3} mol·dm^{-3} Cu(II) + 1 × 10^{-3} mol·dm^{-3} Z + 1 × 10^{-3} mol·dm^{-3} guanine. (a) Z = glycine; (b) Z = bicine; (c) Z = tricine; (d) Z = ADA.

properties of N7 during the formation of the binary nucleobase complexes, is also significant for ternary complexes. Taking into consideration the equilibria which may exist between the open and closed forms of the formed ternary complexes and the effect of the nature of the metal ion coordination in this equilibria, one can account for the observed trend for the stability constants of the different ternary complexes of the type M(II) + NB + Z.

The higher values of stability constants of protonated 1:1:1 ternary complexes containing M(II), guanine, adenine, and the ligands glycine, bicine, or tricine compared with those of the other normal ternary complexes of the type M(II) + NB + Z may be attributed to extra hydrogen bonding of the proton with the oxygen atom of the secondary ligand molecules; this observation may confirm the presence of such extra hydrogen bonding during the formation of the ternary nucleic acid−Cu(II)−protein complexes.

The weaker binding of the glycinate anion by the binary M(II)−nucleobase complexes as compared with that of bicinate or tricinate anions has been observed in some ternary systems [Ni(II) + glycine + guanine, Ni(II) + tricine + guanine, Ni(II) + tricine + uracil, Co(II) + tricine + guanine, Co(II) + bicine + guanine, Co(II) + bicine + thymine, Co(II) + tricine + thymine, Mn(II) + bicine + guanine, Mn(II) + tricine + guanine, Zn(II) + bicine + guanine, Zn(II) + tricine + guanine, Zn(II) + tricine + thymine, Ca(II) + bicine + guanine, and Ca(II) + tricine + guanine]. The fact that the glycinate anion is more basic tends to make it more strongly bound. The effect from the

Table 9. Voltammetric Characteristics[a] of 1 × 10^{-3} mol·dm^{-3} Cu(II) and Its Binary and Ternary Complexes with Zwitterionic Buffer (Z) and Guanine at 25 °C

system	$-E_{pc}$ (mV)	$+E_{pa}$ (mV)	$-(E_{pc} - E_{pa})$ (mV)	I_{pc} (nA)	I_{pa} (nA)
Cu(II)	124.96 ± 2.00	46.08 ± 0.90	78.88	42.13	43.60
Cu(II)(guanine)	172.96 ± 1.80	65.28 ± 1.20	107.68	51.36	47.20
Cu(II)(glycine)	153.76 ± 1.70	57.60 ± 1.80	96.16	15.96	134.63
Cu(II)(bicine)	180.64 ± 1.60	61.44 ± 1.75	119.2	30.39	71.70
Cu(II)(tricine)	219.00 ± 1.90	84.48 ± 1.85	134.52	34.40	34.40
Cu(II)(glycine)(guanine)	141.93 ± 1.80	65.28 ± 1.65	76.65	11.97	128.64
Cu(II)(bicine)(guanine)	200.0 ± 1.60	65.28 ± 1.70	134.72	24.95	45.40
Cu(II)(tricine)(guanine)	249.92 ± 2.00	69.12 ± 1.80	180.8	32.00	31.20

[a] ± uncertainties refer to 3 times the standard deviation in measured potentials.

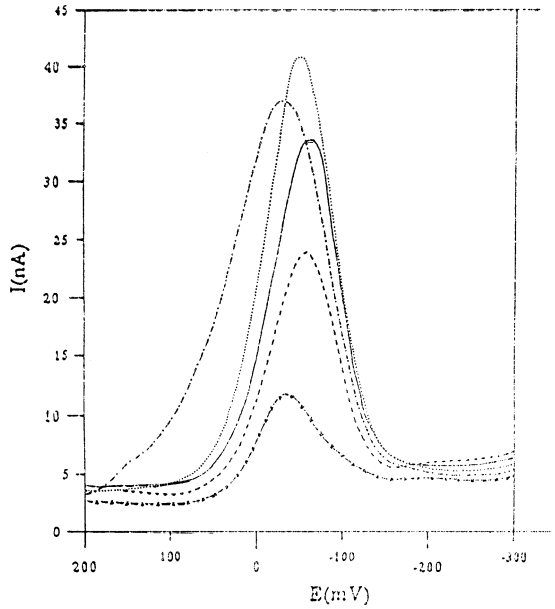


Figure 3. Effect of pH on differential pulse polarograms for the Cu(II) + bicine + guanine system at I = 0.1 mol dm^{-3} KNO_3 and scan rate = 100 mV/s and at 25 °C: (a) pH = 2 (—); (b) pH = 3 (• • •); (c) pH = 4 (- - -); (d) pH = 5 (- • -); (e) pH = 6.5 (- × -).

poorer structural matching between the secondary ligand and the M(II)−nucleobase complex prevails over that from the basicity, and the binding of the glycinate anion secondary ligand by a M(II)−nucleobase complex is weaker than the binding between the bicinate or tricinate anions and the same binary M(II)−nucleobase complex. Also, the participation of the hydroxyl groups of bicinate or tricinate anion during the formation of the mixed ligand complexes may also enhance the stability of the resulting species.

During SUPERQUAD[29] refinement the titration data of the ternary complexes Cu(II)−glycine−adenine, Cu(II)−bicine−adenine, Cu(II)−tricine−adenine, Cu(II)−ADA−adenine, Cu(II)−bicine−guanine, Cu(II)−tricine−guanine, and Cu(II)−ADA−guanine fit satisfactorily on the basis of the monoprotonated ternary complexes in which the binary Cu(II)−Z complexes bind to the nucleobase guanine through its pK_a values which correspond to the N7 deprotonation process. Taking into consideration the high stability of these ternary complexes, especially those containing an adenine moiety, these systems may form a basis for the exploitation of transition metal chelates as a research tool in nucleic acid chemistry and molecular biology. This approach will undoubtedly find applications in chemical tailoring of DNA and RNA or in selective inhibition of gene expression.

Metal ions such as Mg(II), Mn(II), or Zn(II) have an important role in virtually every stage of gene expression involving deoxyribonucleic acid (DNA) replication, transcription, and messenger ribonucleic acid (RNA) translation.[20] Thus, the interaction of our binary and ternary M(II)−NB−Z complexes with double-stranded DNA using a 2-dimensional NMR method is now under consideration through one of our collaborations.

To quantify the stability of ternary complexes relative to the stability of the binary parent complexes, the following equilibrium may be considered

$$\mathrm{M(NB) + M(Z) \rightleftharpoons M(NB)(Z) + M} \qquad (4)$$

The corresponding equilibrium constant is defined by eq 5

$$10^{\Delta \log K_M} = \frac{[\mathrm{M(NB)(Z)}][\mathrm{M(II)}]}{[\mathrm{M(NB)}][\mathrm{M(Z)}]} \qquad (5)$$

Values for Δ log k_M may be calculated according to eq 5.

The results are given in Table 8. It is clearly observed from the table that Δ log k_M values are positive for some of the ternary complexes studied. The higher values for the stability constants of ternary complexes compared with those of the binary systems may be attributed to the interligand interactions or some cooperativity between the coordinate ligands, possibly H-bond formation. This also may be explained on the basis of the π-electron donating tendency of the M(II) ion to the antibonding π^* orbitals of the heteroaromatic N base, such as adenine base, causing strengthing of the M(II)−N bond. Due to the back-donation from metal to adenine base, the d electron content on the metal decreases, which renders the metal more electrophilic. The interaction of the p electrons of the heteroatoms of the secondary ligands with the metal will increase to a greater extent and consequently enhance the formation of the mixed ligand complexes.

The ternary complexes of the type M(II) + NB + Z may be considered as relatively simple models from which information may be gained about the properties of purine or pyrimidine nucleobases regarding the strength of their interactions with the biologically important secondary ligands (glycine, bicine, tricine, or ADA), and even insight into the factors which influence the strength is thus becoming available, as these systems may mimic protein−metal ion−nucleic acid complexes. Our investigation confirmed the formation of ternary complexes of the type M(II) + NB + Z (where Z = glycine, bicine, tricine, or ADA and M(II) = Cu(II), Co(II), Ni(II), Mn(II), Zn(II), Ca(II), or Mg(II) in solution; hence, great reservations should be exercised in employing these biologically important zwitterionic buffer ligands in aqueous solutions in systems containing the above-mentioned metal ions and adenine or hypoxanthine because they will affect the properties of these mucleobases in various ways when they are used as substrates. The study of the systems in the present investigation may lead to guidelines for the synthesis of possible antitumor drugs.

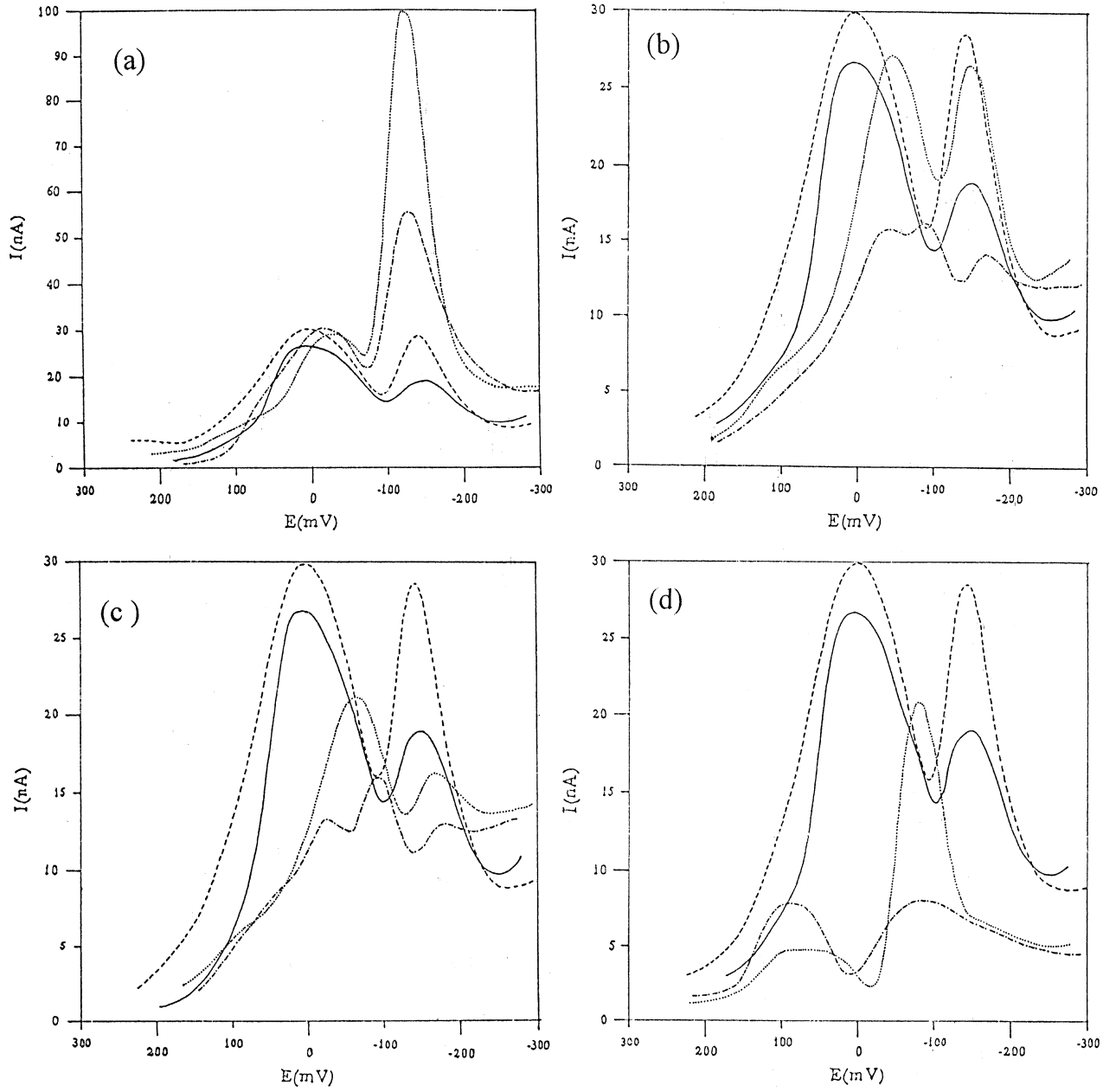


Figure 4. Square wave polarograms for the Cu(II) + Z + guanine system at $I = 0.1$ mol dm^{-3} KNO_3, pH = 6.5, and scan rate = 100 mV/s and at 25 °C: (—) 1×10^{-3} mol·dm^{-3} Cu(II); (• • •) 1×10^{-3} mol·dm^{-3} Cu(II) + 1×10^{-3} mol·dm^{-3} Z; (- - -) 1×10^{-3} mol·dm^{-3} Cu(II) + 1×10^{-3} mol·dm^{-3} guanine; (- • -) 1×10^{-3} mol·dm^{-3} Cu(II) + 1×10^{-3} mol·dm^{-3} Z + 1×10^{-3} mol·dm^{-3} guanine. (a) Z = glycine; (b) Z = bicine; (c) Z = tricine; (d) Z = ADA.

Confirmation of the formation of ternary complexes of the type Cu(II) + guanine + Z (Z = bicine, glycine, tricine, or ADA) in solution has been carried out using differential pulse polarography (DPP), cyclic voltammetry (CV), square wave voltammetry, and UV−visible spectroscopic measurements.

Representative differential pulse polarograms for the systems Cu(II) + bicine + guanine, Cu(II) + guanine, Cu(II) + tricine + guanine, and Cu(II) + ADA + guanine at pH ∼ 6.5 are given in Figure 1. The differential pulse polarograms of the Cu(II) solution show one cathodic peak at $E_p = -68$ mV. This peak may be described as a result of the reduction of Cu^{2+} to Cu (in a two-electron-transfer process) at the glassy carbon electrode.

The addition of primary or secondary ligands caused a slight shift of the cathodic peak to more negative potential, indicating the formation of the binary and ternary complexes in solution. Figure 2 shows the cyclic voltammograms recorded at a glassy carbon electrode for the systems Cu(II) + glycine + guanine, Cu(II) + tricine + guanine, Cu(II) + bicine + guanine, and Cu(II) + ADA + guanine at the scan rate = 100 mV·s^{-1}. It is quite clear that the cyclic voltammograms of the binary and ternary complexes are quite different, confirming the formation of the ternary complexes in solution in accordance with the results obtained by potentiometric pH titrations under the same conditions. The main features of the voltammograms are a two-electron oxidation step at positive potentials and a two-electron reduction step at negative potentials for the Cu(II)−binary and ternary complexes of the above-mentioned types. The cyclic voltammetric behavior is apparently quasireversible. Only in the case of Cu(II) + ADA + guanine was the voltammetric behavior irreversible for the Cu(II) + ADA and Cu(II) + ADA + guanine systems. Figure 3 shows the effect of pH values on the electrochemical reduction of the ternary system Cu(II) + bicine +

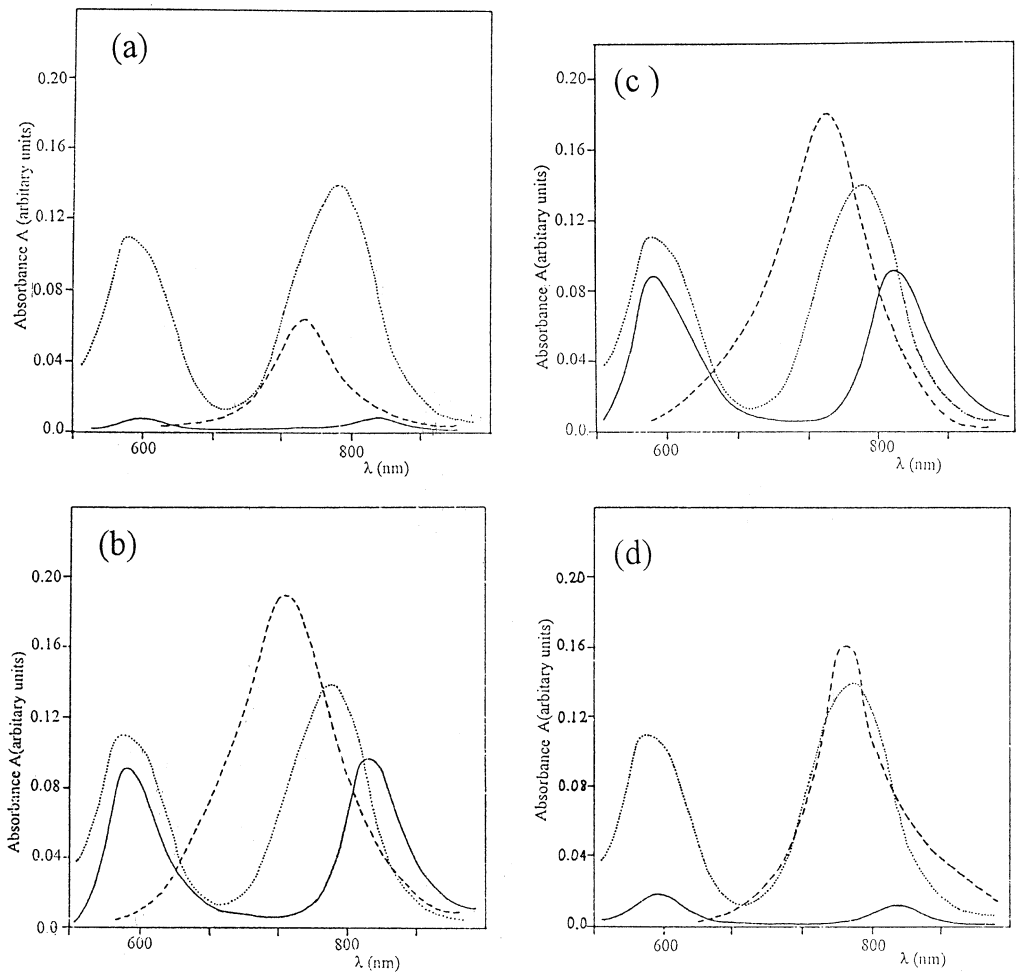


Figure 5. Visible absorbance spectra for the Cu(II) + guanine system at I = 0.1 mol dm^{-3} KNO_3 and at 25 °C: (—) 1 × 10^{-3} mol dm^{-3} Cu(II) + 1 × 10^{-3} mol dm^{-3} Z; (· · ·) 1 × 10^{-3} mol dm^{-3} Cu(II) + 1 × 10^{-3} mol dm^{-3} guanine; (- - -) 1 × 10^{-3} mol dm^{-3} Cu(II) + 1 × 10^{-3} mol dm^{-3} Z + 1 × 10^{-3} mol dm^{-3} guanine. (a) Z = glycine; (b) Z = bicine; (c) Z = tricine; (d) Z = ADA.

guanine. Table 9 contains the cyclic voltammetric parameters for this system, which confirm the presence of ternary complexes at different pH values 2.0−6.5.

The most interesting observation during the square wave voltammetric reduction of the above-mentioned ternary complexes of the type Cu(II) + guanine + Z is the discrimination of the two individual one-electron steps of Cu(II) ⇌ Cu(0) interconversion, giving separate responses for the Cu(II/I) and Cu(I/0) couples. The redox chemistry of Cu(II) is dominated by two-electron conversions between Cu(0) and Cu(II) at a glassy carbon electrode using DPP and CV in this study. Figure 4 shows the square wave voltammograms for 1 × 10^{-3} mol·dm^{-3} Cu(II) in the absence and in the presence of primary and secondary ligands in the above-mentioned ternary complexes. By addition of primary and secondary ligands, a more electronegative peak was observed and the two simple Cu(II) peaks become smaller. The new peaks confirm the formation of ternary Cu(II) + bicine + guanine, Cu(II) + glycine + guanine, and Cu(II) + tricine + guanine in solution. The current of simple Cu(II) is lower than that in the presence of guanine base. An increase in the current was observed on the addition of the ligand, due to the adsorption of Cu(II)−guanine complexes. Then, the current is constant when the surface coverage is reached. Therefore, the shift in peak potential may be due to the complex formation. Further addition of the secondary ligands modified the square wave voltammograms due to the formation of the ternary complexes in solution. On the other hand, the ternary system Cu(II) + ADA + guanine showed similar square wave voltammograms to those of the above-mentioned systems with the exception of a slight positive shift in the potential peaks.

Figure 5 shows the visible absorption spectra of the binary and ternary Cu(II) systems at given pH values. The spectra of the ternary systems are quite different from those of the binary systems, emphasizing the formation of the former in solution.

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