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Coordination properties of Palladium(II) complex taken as a model of an antitumour agent with some selected amino acids, peptides and DNA constituents.

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Abstract

$Pd(DHP)Cl_2$ complex (DHP = 1,3-diamino-2-hydroxopropane), was synthesized and characterized by physico-chemical measurements. The coordination of $[Pd(DHP)(H2O)2]2+$ with some selected bio-relevant ligands as phenylglycine, phenylalanine, lysine, valine, ethanolamine, glycineamide, glycylphenylalanine, glycylleucine, inosine, guanosine and inosine-5'-monophosphate disodium salt was investigated. Stoichiometry and stability constants of the complexes formed are reported at 25 ^{0}C and 0.1M ionic strength. The results show the formation of 1:1 complexes with amino acids. DNA constituents form 1:1 and 1:2 complexes. Peptides form both 1:1 complexes and the coresponding deprotonated amide species. The effect of chloride ion concentration on the formation constant of inosine, taken as a representaive of DNA constituents, complex with $Pd(DPH)^{2+}$ was reporte

KEW WORDS: Coordination property; amino acids; peptides; DNA; 1,3-diamino-2-hydroxopropane and stability constants.

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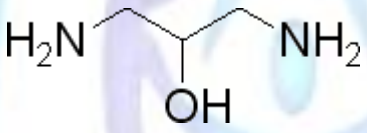
1. Introduction

In 1969, Barnet Rosenberg discovered the cytostatic activity of *cis*- diamminedichloroplatinum(II)- cisplatin- by chance [1]. Cisplatin is nowadays used worldwide in the treatment of testicular and ovarial cancer and is increasingly used against cervical, bladder, and head/neck tumors [2]. Following the example of cisplatin, thousands of platinum –containing compounds have been synthesised and evaluated as potential antitumor drugs but only a few compounds reached clinical applications, viz. carboplatinn or oxaliplatin [3]. The development of new platinum compounds always continues, in the hope to supress side effects, to overcome drug resistance during therapy and to afford a broader range of applications [4]. As known for several years [5], a subsequent reaction with DNA will occur inside the nucleus, which results in a kink in the DNA structure and consequently leads to apoptosis of the cell or to reparation of the DNA by cutting out platinum and resynthesizing at the open sites [6]. This possibility of reparation of the platinated DNA leads to drug resistance and big efforts are still made to create novel platinum-containing compounds to reduce such cisplatin and carboplatin resistence, which may also violate the classical structure-activity relationship [7,8]. Therefore the thermodynamics of the reactions for the Pt(II) complexes is of great interest. In order to avoid the inert substitution behavior of Pt(II) complexes, and on the basis of the remarkable analogy between the coordination chemistry of Pt(II) and Pd(II) complexes, a series of labile Pd(II) complexes have proved useful as models for the Pt(II) complexes.

Work in our laboratories focused on the studies of metal complexes of biological significance [9-15]. Pd(II) complexes with bidentate amine forming five-membered chelate ring were extensively investigated as a model of the antitumor cis-diaminePt(II) complex. The palladium(II) complexes with 1,3-diamino-2-hydroxopropane involves a six-membered chelate ring. The increase in the chelate ring size will increase the bite angle, which have an effect of increasing the steric interaction between the guanines in the cis-Pt(diamine)G_2 adduct, thereby slowing down the rotation of the guanines about the Pt-N_7 bonds [16,17]. Such restrication may stabilize the DNA adduct. The is on line with the finding that cis-Pt(1,4-DACH)Cl_2, (1,4-DACH = 1,4-diaminocyclohexane) where a seven-membered chelate ring is formed, is more active than cisplatin and oxaliplatin in several in *vivo* and in *vitro* tests [18]. Also, the OH group may undergo hydrogen bonding with DNA. Such effects may favor the interaction with DNA, which is the main target for the antitumor agent. For these reasons, it seems therefore of considerable interest to perform a systematic study of the complex formation equilibria between $[Pd(DHP)(H_2O)_2]^{2+}$ and amino acids, peptides, dicarboxylic acids or DNA constituents.



PHD

2. Experimental

2.1. Materials

$PdCl_2$ and 1,3-diamino-2-hydroxopropane (DHP) were provided by Aldrich Chem. Co. The ligands investigated are phenylglycine, phenylalanine, lysine, valine, ethanolamine, glycineamide, glycylphenylalanine, glycylleucine, inosine, guanosine , inosine-5'-monophosphate and 2-mercaptoethylamine . These materials were obtained from Sigma Chemical company and used without further purification. Guanosine, inosine-5'-monophosphate and were prepared in the protonated form with standard HNO_3 solution. Pd(DHP)Cl_2 was converted into the diaqua complex by treating it with two equivalents of $AgNO_3$ as described before [9]. Carbonate- free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution daily. All solutions were prepared in deionized H_2O.

2.2. Synthesis

Pd(PHD)Cl_2 was prepared by dissolving K_2PdCl_4 (2.82 mmol) in 10 ml water with stirring. The clear solution of $[PdCl_4]^{2-}$ was filtered and 1,3-diamino-2-hydroxopropane (2.82 mmol), dissolved in 10 ml H_2O was added drop wise to the stirred solution. The pH was adjusted to 2-3 by the addition of HCl and/or NaOH. A yellowish -brown precipitate of Pd(DHP)Cl_2 was formed and stirred for a further 30 minute at 50 °C. After filtering off the precipitate, it was thoroughly washed with H_2O, ethanol and diethyl ether. A yellow powder was obtained. Anal. Calcd. for $C_3H_{10}N_2OPdCl_2$ (267.3) :C, 13.6; H, 3.7; N, 10.5. Found: C, 13.5; H,4.0; N, 10.3%.

The IR spectrum of the Pd(DHP)Cl_2 complex shows a sharp band at 3414 cm^{-1} for stretching vibration of OH group and distinct bands at 3050 - 3250 cm^{-1}, which can assigned to stretching vibrations of NH_2 group . The complex exhibits a band for (NH_2) bending at 1570 cm^{-1} and a band for the stretching vibration corresponding to Pd-N at 424 cm^{-1}. The ligands in the form of hydrochlorides were converted to the corresponding hydronitrate in the same way as described above

2.3. Apparatus

Potentiometric titrations were performed with a Metrohm 686 titroprocessor equipped with a 665 Dosimat (Switzerland-Herisau). The titroprocessor and electrode were calibrated daily with standard buffer solutions prepared according to NBS specifications at 25.0 ± 0.1^0C [19] and I = 0.1 mol-dm^{-3}, potassium hydrogen phthalate (pH 4.008) and

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a mixture of KH_2PO_4 and Na_2HPO_4 (pH 6.865). A thermostated glass-cell was used equipped with a magnetic stirring system, a Metrohm glass-calomel combined electrode, a thermometric probe and a microburete delivery tube. Elemental microanalyses of the separated solid for C, H and N was performed in the Microanalytical Center, Cairo University. The analyses were performed twice to check the accuracy of the analyses data.

2.4. Procedure and Measuring Techniques

The acid dissociation constants of the ligands were determined by titrating 1 mmole samples of each with standard NaOH solutions. Ligands were converted into their protonated form with standard HNO_3 solutions. The acid dissociation constants of the coordinated water molecules in $[Pd(DHP)(H_2O)_2]^{2+}$ were determined by titrating 1 mmole of complex with standard 0.05 M NaOH solution. The formation constants of the complexes were determined by titrating solution mixtures of $[PdDHP)(H_2O)_2]^{2+}$ (1 mmole) and the ligand in the concentration ratio of 1:1 for amino acids, peptides and dicarboxylic acids and in the ratio of 1:2 (Pd:ligand) for the DNA constituents. The titrated solution mixtures each had a volume of 40 ml and the titrations were carried out at 25 ºC and 0.1 M ionic strength (adjusted with $NaNO_3$), A standard 0.05 M NaOH solution was used as titrant. The pH meter readings were converted into hydrogen ion concentration by titrating a standard acid solution (0.05 mol-dm^{-3}), the ionic strength of which was adjusted to 0.1 mol-dm^{-3}, with standard base solution (0.05 mol-dm^{-3}) at 25 ^{o}C. The pH is plotted against p[H]. The relationship pH - p[H] = 0.05 was observed. $[OH^-]$ value was calculated using a pK_w value of 13.997 [20]. The ionic strength was adjusted to 0.1 mol-dm^{-3} by using $NaNO_3$

The species formed were characterized by the general equilibrium

$$pM + qL + rH \rightleftharpoons (M)_p(L)_q(H)_r$$

for which the formation constants are given by

$$\beta_{pqr} = \frac{[(M)_p(L)_q(H)_r]}{[M]^p[L]^q[H]^r}$$

where M, L and H stand for $[Pd(DHP)(H_2O)_2]^{2+}$ ion, ligand and proton, respectively. The calculations were performed using the computer program [21] MINIQUAD-75. The stoichiometry and stability constants of the complexes formed were determined by trying various possible composition models for the systems studied. The model selected was that which gave the best statistical fit and was chemically consistent with the magnitudes of various residuals, as described elsewhere [21]. Tables (I-IV) list the stability constants together with their standard deviations and the sum of the squares of the residuals derived from the MINIQUAD output. The concentration distribution diagrams were obtained with the program SPECIES [22] under the experimental condition used.

2.5. Spectrophotometric measurements

Spectrophotometric measurements of Pd(DHP)-glycinamide complex were performed by recording the UV-Visible spectra of solutions (A-C) , where (A) = 1mM of $Pd(DHP)(H_2O)_2^{2+}$; (B) = 1 mM of $PdDHP)(H_2O)_2^{2+}$ + 1 mM of glycylglycine + 1 mM of NaOH and (C) = 1mM of $Pd(DHP)(H_2O)_2^{2+}$ + 1 mM of glycylglycine + 2 mM of NaOH. Under these prevailing experimental conditions and after neutralization of the hydrogen ions released, associated with complex formation, it is supposed that the complexes have been completely formed. In each mixture the volume was brought to 10 ml by addition of deionized water and ionic strength is kept constant at 0.1M $NaNO_3$.

3. Results and discussion

3.1. Acid- base equilibria

The acid dissociation constants of the ligands were determined under the experimental conditions of (25 ± 0.1)°C and a constant ionic strength of 0.1 mol-dm^{-3}, which were also used to determine the stability constants of the Pd(II) complexes. The values obtained are consistent with data reported in the literature [23,24].

The acid-base equilibria of the $[Pd(DPH)(H_2O)_2]^{2+}$ complex is characterized by fitting the potentiometric data to various models. The best fit model was found to be consistent with the species 10-1 and 10-2 as given in Eq. (3) and the negative numbers refer to proton loss.

$$[Pd(DHP)(H_2O)_2]^{2+} \rightleftharpoons [Pd(DHP)(H_2O)(OH)]^{+} + H^{+} \quad (3a)$$

100 10-1

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$$[Pd(DHP)(H_2O)(OH)]^{+} \rightleftharpoons [Pd(DHP)(OH)_2] + H^{+} \quad (3b)$$

10-1 10-2

$$\log K_{dimer}$$

$$2\,[Pd(DHP)(H_2O)(OH)]^{+} \rightleftharpoons [Pd(DHP)(OH)_2Pd(deen)]^{2+} \quad (3c)$$

10-1 20-2

The pK_{a1} and pK_{a2} values were found to be 5.61 and 9.92, respectively. The pK_{a1} value is higher than that for $[Pd(AMBI)(H_2O)_2]^{2+}$ (pK_a 4.7) [25] (AMBI = 2-aminomethylbenzimidazole). This shows that the first coordinated water molecule in $[Pd(DHP)(H_2O)_2]^{2+}$ is less acidic than that of $[Pd(AMBI)(H_2O)_2]^{2+}$. This is attributed to the π-acceptor properties of the aromatic moieties of AMBI, which leads to an increase in the electrophilicity of the Pd(II) ion and consequently decreases the pK_a of the coordinated water molecule. The mono-hydroxo- species (10-1) undergoes dimerization as given in Eq. 3. The equilibrium constant for the dimerization reaction can be calculated [14] by equation (4) and amounts to 3.72.

$$\log K_{dimer} = \log \beta_{20-2} - 2 \log \beta_{10-1} \quad (4)$$

3.2. Complex Formation Equilibria Involving Amino Acids

Fitting of pH titration data for Pd(DHP)-amino acid equilibria indicated the formation of 1:1 complexes with high stability constant values . This reveals that amino acids bind through the amino and carboxylate groups. Threonine has an extra binding center on the β-alcoholate group. This group was reported [26,27] to participate in transition metal ion complex formation reactions. The pK_a value of the alcoholate group incorporated in the Pd(II) complex ($\log \beta_{110} - \log \beta_{11-1}$) is 8.71. Therefore in physiological pH (7.4), the –OH group participates in bonding with $Pd(DHP)^{2+}$ ion.

The stability constant of lysine complex is higher than those of simple amino acids. This indicates that lysine coordinates via the two nitrogen centres, i.e. by the two amino groups. This is in line with the strong affinity of Pd(II) ion for nitrogen donor centres. It is to be reported that the bulky groups around the coordination centres as in phenylglycine, phenylalanine and valine has no effect on the stability of the complex.

3.5. Complex Formation Equilibria Involving Peptides

The potentiometric data for the peptide complexes were fitted on the basis of formation of the complexes with stoichiometric coefficients 110 and 11-1, as given in Eq. (5)

$$[Pd(DHP)(H_2O)_2]^{2+} + L^{-} \rightleftharpoons [Pd(DHP)L]^{+} + 2H_2O \quad (5a)$$

100 110

$$K^{H}$$

$$[Pd(DHP)L]^{+} \rightleftharpoons [Pd(DHP)LH_{-1}] + H^{+} \quad (5b)$$

110 11-1

The 110 complex is formed via coordination of the amine and carbonyl groups. On increasing pH, the amide groups undergo deprotonation associated with switch of coordination site from carbonyl to amide and the complex [Pd(DHP(LH$_{-1}$)], (11-1) is formed. Such changes in coordination centres are well documented [26,27]. The pK^{H} of the amide groups incorporated in the Pd^{II} complexes ($\log \beta_{110} - \log \beta_{11-1}$) are in the 3.33-8.32 range. It is interesting to note that the pK_a value for the glycinamide complex is lower than those for other peptides. This can be explained on the basis that the more bulky substituent on the peptide as in glycylleucine and glycylphenylalanine may hinder the structural changes when going from the protonated to the deprotonated complexes. Also, ethanolamine forms the complex species 110 and 11-1, and the logβ110 value for ethanolamine is smaller than those for amino acids. This may be due to the coordination of ethanolamine at low pH through the amino group only. At higher pH, the hydroxyl group undergoes induce ionization and participates in complex formation forming the species 11-1. The pKH value of the coordinated alcohol group in ethanolamine is 5.85. The induce ionization occurs through coordination of the hydroxyl group. Due to the donation of the electron pair on the oxygen to the metal centre, the OH bond is considerably weakened and thus the ionization of the proton occurs at a lower pH.

The distribution diagram for the Pd(DHP)-ethanolamine system is given in Fig. 1. $[Pd(DHP)L]^+$ (110), starts to form at lower pH, its concentration increases with increasing pH and reaches a maximum of 38 % at pH 5.3 . A further increase in pH is accompanied by a decrease in $[Pd(DHP)L]^+$ (110) concentration and an increase in [Pd(DHP)LH-1] (11-1) concentration, reaching a maximum of 100 % at pH 8.7 i.e. in the physiological pH range the deprotonated species (11-1) predominates.

Spectral bands of $Pd(DHP)(H_2O)_2^{2+}$ and its glycinamide complex are quite different in the position of the maximum wavelength and molar absorptivity. The spectrum of $Pd(DHP)(H_2O)_2^{2+}$complex shows an absorption maximum at 354 nm. On the other hand the spectrum obtained for $Pd(DHP)(glycinamide)^{2+}$ (110 species), (mixture B), exhibits a band at 304 nm. The spectrum obtained for $Pd(DHP)(glycinamideH_{-1})^+$ complex (11-1 species), (mixture C) exhibits a band at 282 nm. The progressive shift toward shorter wavelength in the absorption spectrum may be taken as evidence, supporting the potentiometric measurements for the induced ionization of amide upon complex formation.

3.6. Complex Formation Equilibria Involving DNA Constituents

DNA constituents such as inosine, guanosine and inosine-5'-monophosphate form 1:1 and 1:2 complexes with the $Pd(DHP)^{2+}$ ion. However, inosine-5'-monophosphate forms the protonated complexes, in addition to the formation of 1:1 and 1:2 complexes. Inosine-5'-monophosphate forms the mono- and diprotonated complexes (111 and 112). The pKa values of the protonated species values are 4.61 ($\log\beta_{112} - \log\beta_{111}$) and 6.84 ($\log\beta_{111} - \log\beta_{110}$). The former pKa value corresponds to N_1H group and the second pKa value to the $-PO_2(OH)$ group. The N_1H group was acidified upon complex formation by 4.11 pKa units. Acidification of the N_1H group upon complex formation is consistent with previous reports for IMP complex [28]. The phosphate group was not acidified upon complex formation since it is far away formation is explained on the basis of possible hydrogen bonding between posphate group and exocyclic amine group [29].

The concentration distribution diagram for the $Pd(DHP)^{2+}$-IMP system taken as a model for DNA binding , Fig. 2, shows that in the physiological pH range the IMP complex (110) dominates with a maximum concentration of 69 % and the hydrolysed species have no contribution, i.e. the interaction between $Pd(DHP)^{2+}$ and IMP as a DNA constituent is feasible. The protonated species (112) exists in a concentration of 98% at pH 2.0 and the species (111) predominates with the maximum concentration of 88% % at pH 5.8.

3.8. Effect of chloride ion concentration on the equilibrium constants of inosine complex with $[Pd(DHP)(H_2O)]^{2+}$

The data of the effect of chloride ion concentration on the stability of [Pd(DHP)(inosine)] complex was given in Table 3. The stability constant of the 1:1 complex in Pd(DHP)-inosine system tends to decrease with increasing of $[Cl^-]$. This is accounted for on the basis that the concentration of the active species, the diaqua- complex, decrease on increasing $[Cl^-]$, and this in turn will affect the stability of the complexes.

3.9. Displacement reaction of coordinated inosine

The preference of Pd(II) to coordinate to S-donor ligands was previously documented [30,31]. These results suggest that Pd(II)-N adducts can easily be converted into Pd-S adducts. Consequently, the equilibrium constant for such displacement reaction is of biological significance. Consider inosine as a typical DNA constituent (presented by HA) and mercaptoethylamine as a typical thiol ligand (presented by H_2B). The equilibria involved in complex-formation and displacement reactions are:

$$HA \rightleftharpoons H^+ + A^- \qquad (5a)$$

$$H_2B \rightleftharpoons 2H^+ + B^{2-} \qquad (5b)$$

$$[Pd(DHP)]^{2+} + A^- \rightleftharpoons [Pd(DHP)A]^+ \qquad (6a)$$

(100) (110)

$$\beta_{110}^{[Pd(DHP)A]+} = [Pd(DHP)A^+]/[Pd(DHP)^{2+}][A^-] \qquad (6b)$$

$$[Pd(DHP)]^{2+} + B^{2-} \rightleftharpoons [Pd(DHP)B] \qquad (7a)$$

(100) (110)

$$\beta_{110}^{[Pd(DHP)B]} = [Pd(DHP)B]/[Pd(DHP)^{2+}][B^{2-}] \qquad (7b)$$

K_{eq}

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$[Pd(DHP)(A)]^{+} + B^{2-} \rightleftharpoons [Pd(DHP)(B)] + A^{-}$ (8)

The equilibrium constant for the displacement reaction given in eq. (8) is given by

$K_{eq} = [Pd(DHP)(B)][A^{-}]/[Pd(DHP)(A)^{+}][B^{2-}]$ (9)

Substitution from eq. (6b) and (7b) in eq. (9) results in:

$K_{eq} = \beta_{110}^{[Pd(DHP)B]}/\beta_{110}^{[Pd(DHP)A]+}$ (10)

log β_{110} values for $[Pd(DHP)(A)]^{+}$ and $[Pd(DHP)B]$ complexes taken from Table 1 amount to 6.24 and 12.61, respectively, and by substitution in eq. (10) results in log K_{eq} = 6.37. This value clearly indicates how sulfhydryl ligands such as mercaptoethylamine and by analogy glutathione are effective in displacing the DNA constituent, i.e., the main target in tumour chemotherapy.

4. Conclusions

$Pd(DHP)Cl_2$ complex was synthesized and characterized. The interaction of $[Pd(DHP)(H_2O)_2]^{2+}$ with some selected bio-relevant ligands was investigated. Comparing stability constants of Pd(II) complexes with these ligands, it would be possible to evaluate the speciation of Pd(II) complexes in biological fluid. This would form a clear basis for understanding the mode of action of such metal species under physiological conditions. The pK^{H} of the amide groups incorporated in the Pd^{II} complexes has interesting biological implications. Under normal physiological conditions (pH 6-7) the peptide would coordinate to $[Pd(DHP)(H_2O)_2]^{2+}$ in entirely different fashions. Glycylphenylalanine would exist solely in the protonated form, whereas the other peptides would be present entirely in the deprotonated form. Therefore, the slight difference in the side chain of the peptides produces dramatic differences in their behaviour toward the palladium species. The equilibrium constant for displacement reaction of inoosine by mercaptoethylamine measures the deactivation of the Pt/Pd based-drug by the sulphur containing biomolecules.

Antitumour Pt(II)-amine complexes are usually administrated as cis-dichloro-complexes. This form persists in human blood plasma with its high 0.16 M Cl^{-} ion content [32]. The net zero charge on the complex fasters its passage through cell walls. Within many cells the Cl^{-} ion concentration is much lower, only 4mM. Under this low chloride ion concentration, the reactivity of the Pt(II)-amine complex increases. Therefore a realistic extrapolation of the present study to biologically relevant conditions will require investigating the effect of $[Cl^{-}]$ on the stability constant of the complexes

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Table 1. Formation constant of $M_pL_qH_r$ species in aqueous solution at 25 ± 0.1 ^oC and I= 0.1 mol-dm^{-3}($NaNO_3$).

System	p	q	r[a]	logβ[b]	S[c]
Pd(DHP)$(OH_2)_2$	1	0	-1	-5.61(0.01)	1.1E-7
	1	0	-2	15.53(0.03)	
	2	0	-2	-7.50(0.02)	
Phenylglycine	0	1	1	9.01 (0.01)	3.4E-8
	0	1	2	11.47(0.01)	
	1	1	0	9.80(0.02)	4.2E-7
Phenylalanine	0	1	1	9.12 (0.01)	7.5E-8
	0	1	2	11.01(0.01)	
	1	1	0	9.74(0.01)	1.2E-8
Valine	0	1	1	9.64(0.01)	6.7E-8
	0	1	2	11.96(0.01)	
	1	1	0	10.49(0.01)	9.5E-8
Threonine	0	1	1	9.06(0.01)	2.7E-7
	0	1	2	11.03(0.02)	
	1	1	0	9.12(0.02)	2.1E-7
	1	1	-1	0.41(0.03)	
Lysine	0	1	1	10.44(0.01)	1.4E-8
	0	1	2	19.66(0.01)	
	1	1	0	10.39(0.03)	5.1-8
	1	1	1	20.30(0.02)	

Ethanolamine	0	1	1	9.31 (0.01)	6.0E-8
	1	1	0	6.94(0.02)	8.8E-8
	1	1	-1	1.09 (0.01)	
Mercaptoethylamine	0	1	1	10.85(0.01)	4.9E-8
	0	1	2	19.45(0.01)	
	1	1	0	12.61(0.05)	2.1E-7
	1	1	1	18.01(0.07)	
Glycinamide	0	1	1	7.89(0.01)	2.9E-8
	1	1	0	7.77(0.08)	5.3E-7
	1	1	-1	4.44(0.02)	
Glycylphenylalanine	0	1	1	8.24(0.00)	2.0E-8
	0	1	2	11.70(0.01)	9.7E-7
	1	1	0	6.63(0.03)	
	1	1	-1	-1.69(0.08)	
Gylcylleucine	0	1	1	8.12(0.02)	4.1E-8
	1	1	0	7.23(0.03)	6.3E-7
	1	1	-1	1.19 (0.04)	
Inosine	0	1	1	8.43 (0.01)	4.1E-8
	1	1	0	6.24(0.02)	4.8E-9
	1	2	0	9.81(0.07)	
Guanosine	0	1	1	9.13(0.01)	2.8E-8
	1	1	0	8.82(0.08)	2.0E-7
	1	2	0	16.75(0.10)	
Inosine-5'-monophosphate	0	1	1	8.72(0.01)	4.3E-8
	0	1	2	14.71(0.02)	
	1	1	0	7.72(0.03)	3.3E-8
	1	2	0	11.31(0.04)	
	1	1	1	14.56(0.03)	
	1	1	2	19.17(0.04)	

[a] p, q and r are the stoichiometric coefficients corresponding to $Pd(DHP)^{2+}$, (amino acid, dicarboxylic acid, peptide or DNA) and H^+, respectivelyStandard deviations are given in parentheses, [c] Sum of square of residuals.

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Table 2. Effect Chloride Ion Concentration on the formation constant of Pd(DHP)-Inosine complex at 25 ^{0}C and 0.30M ionic strength.

[Cl-] ion Concn.	P	q	r[a]	log β[b]	S[c]
0.00M	0	1	1	8.53(0.01)	1.2E-8
	1	0	-1	-5.20(0.01)	5.3E-8
	1	0	-2	-14.67(0.01)	
	1	1	0	7.10(0.03)	5.9E-6
	1	2	0	11.35(0.05)	
0.05 M	1	0	-1	-7.41(0.01)	4.8E-8
	1	0	-2	-16.99(0.01)	
	1	1	0	6.65(0.01)	1.5E-8
	1	2	0	11.16(0.03)	
0.10 M	1	0	-1	-7.89(0.01)	8.2E-8
	1	0	-2	17.70(0.01)	
	1	1	0	6.51(0.01)	1.2E-8
	1	2	0	10.89(0.02)	
0.15 M	1	0	-1	-8.28(0.01)	5.6E-8
	1	0	-2	-18.10(0.02)	
	1	1	0	6.35(0.01)	7.7E-7
	1	2	0	10.84(0.02)	
0.20 M	1	0	-1	-8.34(0.01)	8.3E-8
	1	0	-2	-18.23(0.01)	
	1	1	0	6.19(0.01)	1.6E-9
	1	2	0	10.50(0.01)	
0.25 M	1	0	-1	-8.51(0.01)	1.5E-7
	1	0	-2	-18.45(0.01)	
	1	1	0	6.09(0.03)	6.0E-7
	1	2	0	10.48(0.01)	
0.30 M	1	0	-1	-8.53(0.01)	1.5E-7
	1	0	-2	-18.46(0.03)	
	1	1	0	6.03(0.01)	3.2E-7
	1	2	0	10.41(0.01)	

[a]p,q and r are the stoichiometric coefficients corresponding to Pd(DHP), Inosine and H^{+} respectively.

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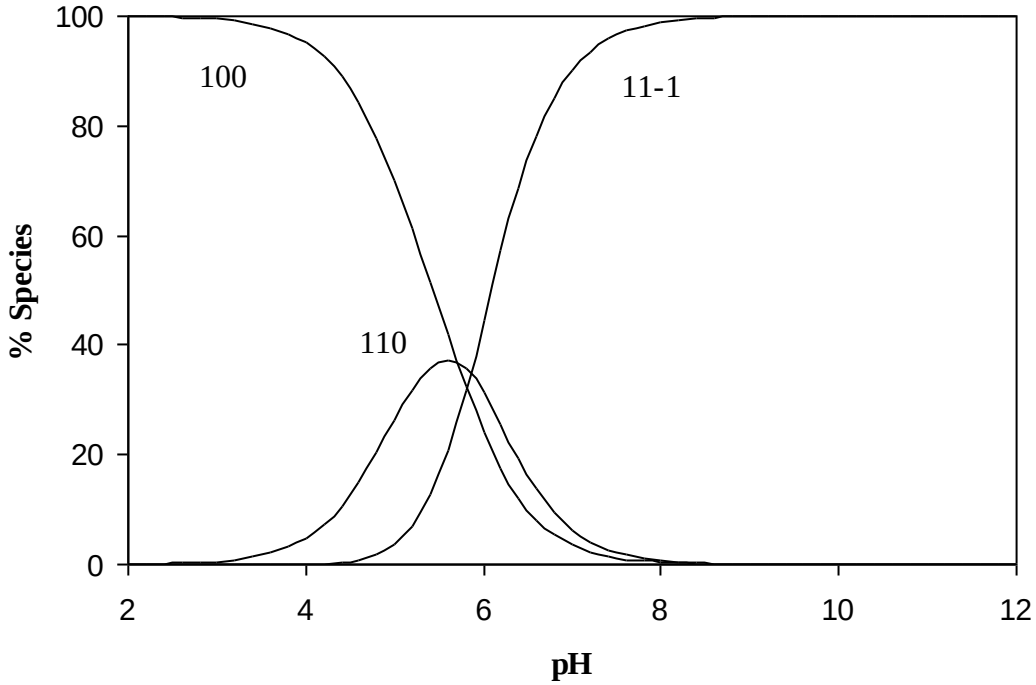

Fig. 1. Concentration distribution of various species as a function of pH in the $Pd(DHP)^{2+}$-ethanolamine system

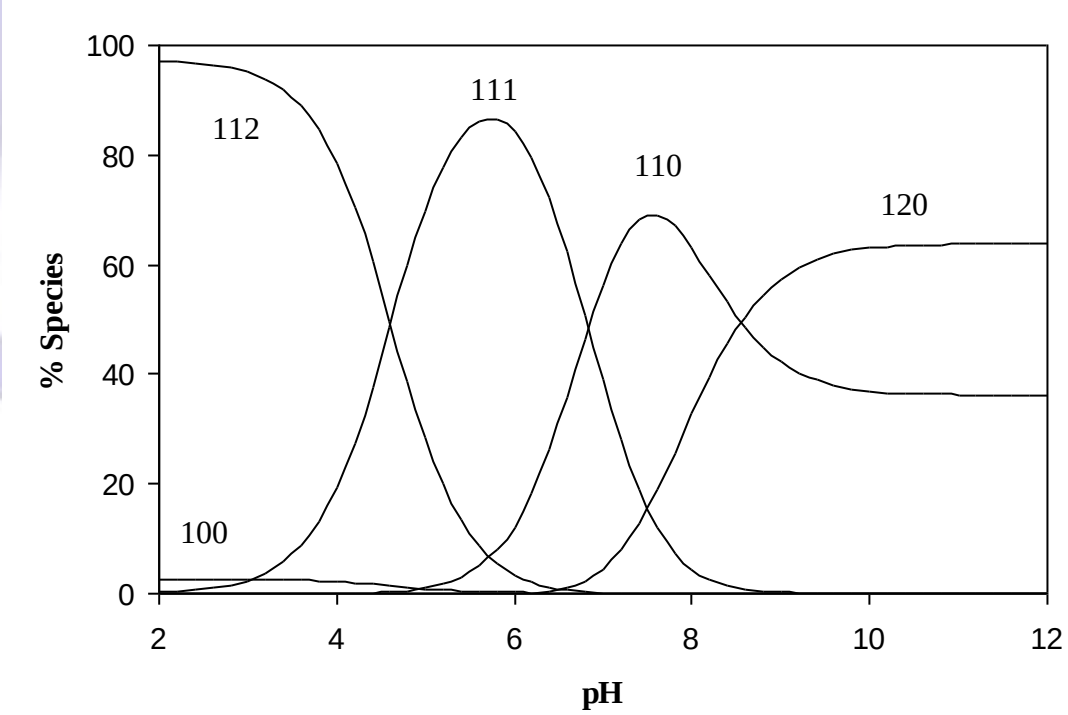

Fig. 2. Concentration distribution of various species as a function of pH in the $Pd(DHP)^{2+}$-IMP system.