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Complex formation reactions and equilibrium studies of mixed ligand complexes of diaqua (1-Phenyl piperazine)(Palladium)(II) with some biologically relevant ligands

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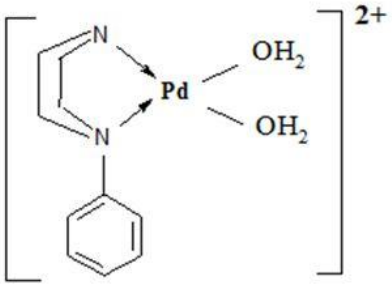
Abstract

With the purpose of the search of new antitumor metal complexes, the complex-formation reactions of $[Pd(Phenpip)(H_2O)_2]^{2+}$ (Phenpip = 1-Phenylpiperazine) with some selected bio-relevant ligands, containing different functional groups (amino acids, peptides, DNA constituents and dicarboxylic acids) were investigated. Stoichiometry and stability constants for the complexes formed are reported. The results show the formation of 1 : 1 complexes with amino acids and dicarboxylic acids. The effect of chelate ring size of the dicarboxylic acid complexes on their stability constants was examined. Peptides form both 1 : 1 complexes and the corresponding deprotonated amide species. Structural effects of the peptide on the amide deprotonation were investigated. DNA pyrimidinic constituents, such as uracil, uridine, thymidine and thymine, form 1 : 1 and 1 : 2 complexes, whereas purinic constituents, such as inosine, inosine 5´-monophosphate (5-IMP) form only 1 : 1 complexes. The stability constant of the complexes formed in solution were determined and the binding centers of the ligands were assigned. The concentration distribution diagrams of the complexes were evaluated

Keywords: *Pd(II) complexes, 1-Phenyl piperazine, bio-relevant ligands, Stability constant*

1 Introduction

Metal compounds are widely used as drugs, one of the major employment domains being the cancer therapy [1,2]. Even if cisplatin is the most successful metal-based drug, an extensive number of tumor-inhibiting metal complexes, with diverse mechanisms of action, were synthesized and reported recently [3]. The great majority of antitumor metal complexes synthesized and characterized have been structural analogs of cisplatin. However, there has been a leveling off or perhaps even a decrease in the number of new compounds of this type, possibly because it is beginning to transpire that substantial advances are unlikely to be made with these compounds. the severe toxic side effects including nephrotoxicity, neurotoxicity,and emetogensise limit the dose that can be given to patients [4]. At the same time there has been an emergence of new structural types of metallic complexes often with promising activity and able to circumvent cisplatin resistance. The similarity between the coordination chemistry of platinum(II) and palladium(II) compounds supports the theory that palladium complexes can act successfully as antitumor drugs. Several studies demonstrate that palladium derivates exhibit a noticeable cytotoxic activity, similarly to standard platinum-based drugs (e.g. cisplatin, carboplatin and oxaliplatin) used as reference, and show fewer side effects relative to other heavy metal anticancer compounds [4]. Recent work in our laboratories focused on equilibria of complex formation reactions of cis-(diamine)palladium(II) complexes with DNA, the major target in chemotherapy of tumors, and amino acids, peptides, and dicarboxylic acids and esters [5–10]. In this project, complex formation equilibria between $[Pd(Phenpip)(H_2O)_2]^{2+}$ (structure 1) and some selected bio-relevant ligands are investigated. The study of 1-phenyl piperazine complexes was performed because the piperazine ring may undergo stacking interactions with the sugar group of DNA. This will enhance complex formation with DNA subunits, which is a principal target in the chemotherapy of tumors [11]. The latter effect is similar to that reported for carboplatin, where the stacking interaction between the cyclobutane ring and the sugar group is part of the increased antitumor activity [12].



Structure 1: Diaqua (1-Pheny1 piperazine)(Palladium)(II)

2 Experimental

2.1 Materials:

$PdCl_2$ was obtained from Aldrich, 1-phenyl piperazine was from Sigma . Amino acids, glycine, alanine, phenylalanine, DL-valine, DL-proline, ornithine, L-histidine, histamine dihydrochloride, methylamine, imidazole, , S-methylcystine and methionine were provided by Sigma Chemical Co. The peptides used (glycinamide, glycylglycine, asparagine, and glutamine) and the dibasic acids used (cyclobutane dicarboxylic acid, malonic, oxalic, succinic, and adipic acid) were all provided by BDH Biochemicals Ltd, Poole, England. The DNA constituents (uracil, uridine, thymine, thymidine, inosine and inosine-5'-monophosphate, were provided by Sigma Chemical Co. Ligands in the form of hydrochlorides were converted into the corresponding hydronitrates. The nucleotides were prepared in the protonated form with standard HNO_3 solution. All solutions were prepared in deionized water.

[Pd(Phenpip)Cl_2] was prepared by heating $PdCl_2$ (0.177 g, 1.0mM) and KCl (0.149 g, 2.0mM) in 10 mL water to 70°C with stirring. The clear solution of $[PdCl_4]^{2-}$ was filtered and the ligand 1-phenyl piperazine (0.162 g, 1.0mM) dissolved in 10 mL H_2O was added dropwise to the stirred solution. The pH value was adjusted to 2–3 by the addition of HCl and/or NaOH. A yellowish-brown precipitate of [Pd(Phenpip)Cl_2] was formed. The precipitate was stirred for an additional 30 min at 60°C. After filtering off the precipitate, it was thoroughly washed with H_2O, ethanol, and diethyl ether. Yellow powder was obtained. Analysis calculated for $C_{10}H_{14}N_2PdCl_2$: Calc (%) C, 35.3; H, 4.12; N, 8.2. Found (%) C, 35.1; H, 4.22; N, 8.4

The dichlorocomplex [Pd(Phenpip)Cl_2] was converted into the corresponding aqua complex [9] in solution by the addition of two equivalents of $AgNO_3$, heating to 40–50°C for 2 h, and removing the precipitated AgCl by filtration.

2.2 Apparatus

Potentiometric titrations were performed with a Metrohm 686 titroprocessor equipped with a 665 Dosimat. The titroprocessor and electrode were calibrated with standard buffer solutions, prepared according to NBS specification [13]. All titrations were carried out at 25.0° C ± 0.1 in purified nitrogen using a titration vessel described previously [6].

2.3 Procedure and measuring technique

Acid dissociation constants of the ligands were determined by titrating 0.20mM samples of each with standard NaOH solutions. Ligands were converted into their protonated form with standard HNO_3 solutions. Acid dissociation constants of the coordinated water in $[Pd(Phenpip)(H_2O)_2]^{2+}$ were determined by titrating 0.20mM of complex with standard 0.05M NaOH solution. The formation constants of the complexes were determined by titrating solution mixtures of $[Pd(Phenpip)(H_2O)_2]^{2+}$ (0.20mM) 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 DNA constituents. The titrated solution mixtures each had a volume of 40mL and the titrations were carried out at 25ºC and 0.1M ionic strength (adjusted with $NaNO_3$). A standard 0.05M NaOH solution was used as a titrant. The equilibrium constants for the species of the general formula $M_lL_pH_q$ (M = [Pd(Phenpip)], L = amino acid, dicarboxylic acids, peptide or DNA constituent), were calculated using the non-linear least square computer program[17] MINIQUAD-75 [14]. The stoichiometry and stability constants of the complexes formed were determined by trying various possible composition models. The model selected gave the best statistical fit and was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals [14]. The stability constants together with their standard deviations (SDs) and the sum of the

squares of the residuals derived from the MINIQUAD output are summarized in Tables 1 to 4. The species distribution diagrams were obtained using the program SPECIES[15**]** under the experimental conditions employed.

3 Results and discussion

The acid dissociation constants of the ligands were determined at 25ºC and constant 0.10M ionic strength (adjusted with $NaNO_3$), as were the stability constants of the Pd(II) complexes. The results obtained are in good agreement with literature data [6, 16].

3.1 Acid–base equilibria of [Pd(Phenpip)(H2O)2]$^{2+}$

The complex $[Pd(Phenpip)(H_2O)]^{2+}$ ion may undergo protolysis depending on the pH of the solution. Its acid–base chemistry was characterized by fitting the potentiometric data to various acid–base models. The best fit model was found to be consistent with species of the composition 10–1 and 20-1, as given in Eqs.1 and 2 .

$$\underset{100}{[Pd(Phenpip)(H_2O)_2]^{2+}} \rightleftharpoons \underset{10\text{-}1}{[Pd(Phenpip)(OH)(H_2O)]^{+}} + H^{+} \quad (1)$$

$$\underset{10\text{-}1}{[Pd(Phenpip)(OH)(H_2O)]^{+}} \rightleftharpoons \underset{10\text{-}2}{[Pd(Phenpip)(OH_2)]} + H^{+} \quad (2)$$

The pK_{a1} and pK_{a2} values were found to be 6.2 and 8.1, respectively. The concentration of the monohydroxo species 10–1 increases with increasing pH, and predominates in the pH range 6 to 8, with formation percentages of *ca.* 80% (the distribution diagram is shown in Fig. 1 . A further increase in pH is accompanied by an increase in the concentration of the dihydroxo species, which is the main species present in solution above a pH of *ca.* 9. This reveals that in the physiological pH range, that is, at pH 6–7, the monohydroxo complex (10-1) predominates and can interact with the DNA subunits. At higher pH, the dihydroxo complex (10-2) will be the major species. This complex is less active compared to the monohydroxo complex, and consequently the ability of DNA to bind the Pd(amine) complex will decrease significantly.

3.2 Complexes involving amino acids

The analysis of the titration data for the Pd(Phenpip)-amino acid system showed the formation of 1 : 1 complexes with stability constants larger than for the corresponding monodentate methylamine complex (Table 1). This indicates that amino acids bind through the amino and carboxylate groups.

Phenylalanine forms a more stable complex than alanine, although the amino group of the Phenylalanine is less basic than that of alanine. This may be due to some stacking interactions between the phenyl group of phenylalanine and the phenpip ligand . This will contribute to the stabilization of the formed complex.

The stability constants of histidine, and ornithine complexes are higher than those of simple amino acids. This indicates that these amino acids coordinate via the two nitrogen centres, that is, imidazole and amino groups in the case of histidine, and by two amino groups in the case of ornithine. This formulation is supported by the high affinity of Pd(II) to nitrogen donor centers. They also form, in addition to 1:1 complexes, the monoprotonated species. The pk_a of the protonated complex was calculated using equation 3.

$$pk_a = \log \boldsymbol{\beta}_{111} - \log \boldsymbol{\beta}_{110} \quad (3)$$

The pk_a values of the protonated species are 2.89 for histidine, 6.81 for ornithine. Although *S*-Methylcysteine has the lowest pK_a value (8.51) among the studied amino acids, its complex has a higher stability constant than that for amino acids such as glycine. This may be taken as evidence that the sulfur atom is participating in the complex formation process. It is also interesting to note that , *S*-methylcysteine forms a more stable complex than methionine. This may be explained based on the fact that the five membered chelate ring in the former complex is energetically more favoured than the six-membered chelate ring in the latter complex. The species distribution diagram of Pd(Phenpip)-alanine complex taken as an example of amino acids is Fig 2. The (110) species starts to form at pH 2.0, and with increasing of pH its concentration increases reaching ≈ 78% between 6-8. This means that the 110 species predominates in the physiological pH range.

3.3 Complexes involving peptides

For the Pd(Phenpip)–peptide system the potentiometric data were fitted to various models. The most acceptable model was found to be consistent with the formation of complexes with stoichiometric coefficients 110 and 11-1 according to Eqs. (4) and (5).

$$[Pd(Phenpip)(H_2O)_2]^{2+} + L^- \underset{}{\overset{K}{\rightleftharpoons}} \underset{110}{[Pd(Phenpip)L]^+} + 2\,H_2O \quad (4)$$

$$[Pd(Phenpip)L]^+ \overset{K^H}{\rightleftharpoons} \underset{11\text{-}1}{[Pd(Phenpip)LH_{-1}]} + H^+ \quad (5)$$

The 110 complex is formed *via* coordination of the amino and carbonyl groups. Upon deprotonation of the amide group, the coordination sites could switch from the carbonyl oxygen to the amide nitrogen with release of the amide hydrogen forming the complex such that the 11-1 complex is formed. Such changes in coordination modes are well documented. The pK^H values of the amide groups incorporated in the Pd(II) complexes ($\log \beta_{110} - \log \beta_{11\text{-}1}$) are in the 4.15–7.80 range (Table 2) . It is noteworthy that the pK^H for glycinamide complex is lower than that of other peptides. This signifies that the more bulky substituent group on the peptide may serve to hinder the structural change in going from the protonated to the deprotonated complexes. The pK^H of the glutamine complex is markedly higher than that for other peptide complexes. This is ascribed to the formation of a seven-membered chelate ring, which would probably be more strained and therefore less favoured. The relative magnitude of the pK^H values of the Pd(II) complexes with peptides has interesting biological implications. Under normal physiological conditions (pH 6-7) the peptide would coordinate to $[Pd(Phenpip)(H_2O)_2]^{2+}$ in entirely different fashions. Glutaminate would exist solely in the protonated form, whereas the other peptides would be present entirely in the deprotonated form. In addition, the slight difference in the side chain of the peptides produces dramatic differences in their behaviour towards the palladium species.
The speciation diagram of glycylglycine complex is given in Fig 3. The $Pd(Phenpip)(L)^+$ (110) species starts to form at pH 2.0, and with increasing of pH its concentration increases reaching ≈ 82% at pH 5.5. Further increase of pH is accompanied by a decrease in $Pd(pip)(L)^+$ concentration and an increase of $Pd(pip)(LH_{-1})$ formation.

3.4 Complexes involving dicarboxylic acid

The potentiometric data of Pd(Phenpip)-dicarboxylic acid complexes is best fitted considering formation of the 1 : 1 species and its protonated form. The results in Table 3 show that oxalic acid complex forming a five-membered chelate ring is most stable, whereas adipic acid complex is the least stable as the complex involves the formation of the least stable eight-membered chelate ring. The pK_a values of the protonated species for $[Pd(pip)HL]^{]+}$ are in the range 3.6–4.7, lower than those for HL^- . The lowering of the pK_a is due to the acidification of the second carboxylic acid group upon coordination of Pd(II) to one carboxylate group [6]. The concentration distribution diagram of the oxalic acid complex is given in Fig. 4. The monoprotonated species attains its maximum concentration of 98% around pH ≈ 3.0. This form has one coordination site available for binding to DNA. Such species was documented to be the active form in the case of carboplatin [12].

3.5 Complexes involving DNA constituents

DNA constituents such as the pyrimidines uracil, uridine, thymine, and thymidine have basic nitrogen donors (N_3) [17] as reflected from the high pK_a values of pyrimidines (pK_a> 9). They form 1 : 1 and 1 : 2 complexes. The results are presented in Table 4. The complexes are predominating above pH 8. The thymine complex is more stable than that of uridine, probably due to the high basicity of the N_3 group of thymine resulting from the extra electron-donating methyl. Inosine has two coordination sites, N_1H and N_7H. The pK_a value of N_7H is too low to determine by potentiometry. The pK_a of N_1H group is 8.43, in agreement with that obtained previously [18]. Inosine-5´-monophosphate has, in addition, a phosphate as binding site. The pK_a of the phosphate is 6.32. This value compares favorably with recently published data for phosphates; the pK_a value of phosphate group of adenosine-triphosphate is 6.21 [19]. Inosine and its nucleotides inosine-5'-monophosphate form the monoprotonated complex, in addition to the formation of 1 : 1 and 1 : 2 complexes.

The pK_a of the protonated inosine complex is 5.87; this value corresponds to N_1H. The lowering of this value with respect to that of free inosine (pK_a=8.43) is due to acidification under the effect of complex formation [20, 21].

4 Conclusion

The present investigation describes the formation equilibria of $[Pd(Phenpip)(H_2O)_2]^{2+}$ with ligands of biological significance. The results show that amino acids form highly stable complexes, the substituent on the α-carbon atom has a significant effect on the stability of the formed complex. The thioether group in *S*-methylcysteine increases the stability constant of its complex as a result of the stronger donor properties of the sulfur atom. The imidazole group in histidine increases the stability of the complex due to high affinity of Pd^{II} for the nitrogen donor group.

The present study shows clearly that the $[Pd(Phenpip)(H_2O)_2]^{2+}$ complex can form strong bonds with peptides and promote facile deprotonation of the peptide. The relative magnitudes of the pK_a values of the Pd(II) complexes with peptides have interesting biological implications. Under normal physiological conditions (pH 6–7), the peptides would coordinate to $[Pd(Phenpip)(H_2O)_2]^{2+}$ in entirely different ways. Glutaminate exists solely in its protonated form, whereas the other peptides are present entirely in the deprotonated form. Also, slight differences in the side chain of the peptides seem to produce dramatic differences in their behaviour toward the Pd(II) complex.

Among the dicarboxylic acid ligands it may be concluded that, CBDCA forms the most stable complex, which is inconsistent with the fact that CBDCA complexes have the highest anti-tumour activity. In addition, CBDCA forms the ring opened mono-protonated complex which may interact with DNA, *i.e.* the main target for antitumour.

From a combination of stability constant data of such diaqua complexes with dicarboxylic acids, amino acids, peptides and DNA constituents, it will in principle be possible to calculate the equilibrium distribution of the metal species in biological fluids where all types of ligands are present simultaneously.

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Table 1: Formation constants for complexes of $[Pd(Phenpip)(H_2O)_2]^{2+}$ with amino acids at 25ºC and 0.1 M ionic strength

System	**M**	**L**	**H[a]**	**Logβ[b]**	**S[c]**
$[Pd(Phenpip)]^{2+}$-OH	1	0	-1	-6.21(0.01)	1.67E-8
	1	0	-2	-14.34(0.03)	
Glycine	0	1	1	9.60 (0.01)	1.6E-7
	0	1	2	11.93 (0.02)	4.5E-7
	1	1	0	9.89(0.04)	
Alanine	0	1	1	9.96 (0.01)	9.3E-8
	0	1	2	11.89 (0.007)	1.349E-7
	1	1	0	10.25(0.03)	
Phenylalanine	0	1	1	9.12 (0.01)	9.3E-8
	0	1	2	11.01 (0.03)	8.3E-8
	1	1	0	10.86(0.10)	
DL-Valine	0	1	1	9.57 (0.01)	9.9E-8
	1	1	2	11.70 (0.03)	7.6E-8
	1	1	0	8.77 (0.03)	
Ornithine	0	1	1	10.58 (0.01)	7.0E-8
	0	1	2	19.432 (0.02)	
	0	1	3	21.39 (0.03)	2.3E-7
	1	1	0	13.39 (0.03)	9.3E-8
	1	1	1	20.20(0.02)	
L-Histidine	0	1	1	9.53 (0.01)	1.8E-8
	0	1	2	15.81 (0.01)	
	1	1	0	12.72 (0.03)	1.66E-7
	1	1	1	15.61(0.02)	
DL-Proline	0	1	1	10.52 (0.01)	4.4E-8
	1	1	2	12.03 (0.03)	
	1	1	0	10.53(0.05)	6.3E-8
Imidazole	0	1	1	7.04 (0.01)	1.7E-8
	1	1	0	6. 47(0.04)	7.097E-9
	1	2	0	11.96 (0.03)	
Methylamine	0	1	1	10.03 (0.04)	4.4E-7
	1	1	0	8.21 (0.08)	2.94E-8
	1	2	0	15.74 (0.10)	
Histamine	0	1	1	9.88(0.01)	2.4E-8
	1	1	2	15.97(0.01)	1.76E-8
	1	1	0	11.196(0.13)	
S-Methylcystine	0	1	1	8.51(0.01)	6.4E-7
	1	1	0	10.73(0.01)	9.4E-7
	1	1	0	11.19 (0.13)	
Methionine	0	1	1	9.18(0.01)	3.4E-8
	0	1	2	11.08(0.01)	5.76E-8
	1	1	0	9.19 (0.13)	

Table 2: Formation constants for complexes of $[Pd(Phenpip)(H_2O)_2]^{2+}$ with peptides at 25ºC and 0.1M ionic strength.

System	M	L	H[a]	Logβ[b]	S[c]
Glycinamide	0	1	1	7.88 (0.00)	
	1	1	0	7.08(0.05)	1. 7E-8
	1	1	-1	3. 93(0.05)	2.4E-6
Glycylglycine	0	1	1	7.94 (0.01)	1.6E-7
	1	1	0	7.55(0.028)	3.6E-8
	1	1	-1	1.04 (0.05)	
L-Glutamine	0	1	1	7.96 (0.01)	7.3E-8
	1	1	0	8.87 (0.02)	5.6E-8
	1	1	-1	1.04(0.08)	
DL-Aspargine	0	1	1	8.55 (0.02)	9.3E-8
	1	1	0	8.93(0.031)	6.9E-8
	1	1	-1	1. 97(0.049)	

Table 3: Formation constants for complexes of $[Pd(Phenpip)(H_2O)_2]^{2+}$ with dibasic acids at 25ºC and 0.1M ionic strength

System	M	L	H[a]	Logβ[b]	S[c]
Oxalic acid	0	1	1	4.10(0.01)	1.5E-8
	0	1	2	5.78(0.06)	
	1	1	0	5.04(0.14)	2.4E-7
	1	1	1	9.74(0.14)	
Malonic acid	0	1	1	5.42(0.01)	2.7E-8
	0	1	2	8.19(0.06)	
	1	1	0	5.01(0.04)	3.7E-7
	1	1	1	9.32(0.14)	
1,1-Cyclobutanedicarboxylic acid (CBDCA)	0	1	1	5.54(0.01)	1.6E-7
	0	1	2	8.77(0.06)	
	1	1	0	5.56(0.02)	8.6E-8
	1	1	1	9.80 (0.101)	
Succinic acid	0	1	1	5.35(0.01)	9.3E-8
	0	1	2	9.42(0.02)	
	1	1	0	4.01 (0.02)	1.3E-7
	1	1	1	7.98 (0.02)	
Adipic acid	0	1	1	5.23(0.01)	9.3E-8
	0	1	2	9.61(0.01)	
	1	1	0	3.88 (0.03)	8.3E-8
	1	1	1	7.55 (0.06)	

Table 4: Formation constants for complexes of $[Pd(Phenpip)(H_2O)_2]^{2+}$ with DNA constituents at 25ºC and 0.1M ionic strength.

System	M	L	H[a]	Logβ[b]	S[c]
	0	1	1	9.28 (0.05)	1. 7E-8
Uracil	1	1	0	8.13 (0.02)	3.5E-8
	1	2	0	14. 84(0.04)	
	0	1	1	9.01 (0.01)	1.6E-7
Uridine	1	1	0	8. 39(0.034)	8.6E-8
	1	2	0	14.90(0.06)	
Thymine	0	1	1	9.58 (0.01)	9.3E-8
	1	1	0	8.79(0.034)	1. 49E-7
	1	2	0	15.20(0.06)	
Thymidine	0	1	1	9.55 (0.01)	9.3E-8
	1	1	0	8.52(0.034)	1. 9E-7
	1	2	0	15.34(0.06)	
Inosine	0	1	1	8.43 (0.02)	9.3E-8
	1	1	0	6.03(0.03)	
	1	2	0	11.49(0.04)	8.3E-8
	1	1	1	11.90(0.01)	
Inosine-5´-monophosphate	0	1	1	8.95 (0.02)	9.3E-8
	0	1	2	15.27(0.02)	
	0	1	3	17.10(0.06)	8.3E-8
	1	1	0	8.33(0.031)	
	1	2	0	13.49 (0.04)	
	1	1	1	14.30(0.06)	

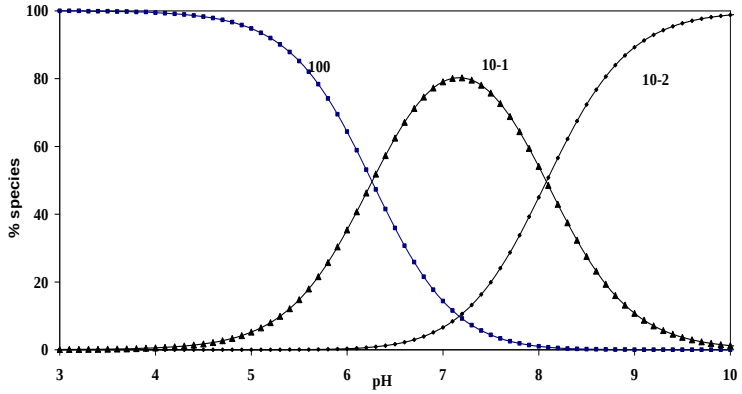

Figure 1. Concentration distribution of various species as a function of pH in the hydrolysis of $[Pd(Phenpip)(H_2O)_2]^{2+}$ complex system (at concentration of 1.25mML $^{-1}$ for Pd(Phenpip).

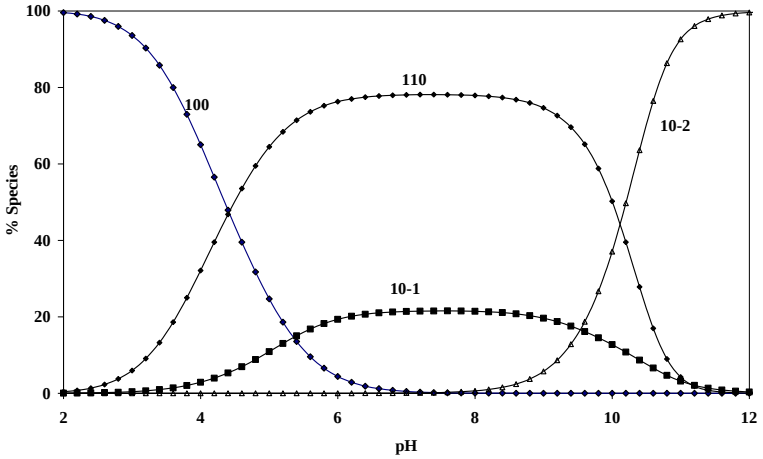

Figure 2. Concentration distribution of various species as a function of pH in the Pd(Phenpip)-alaninesystem (at concentration of 1.25mML^{-1} for Pd(Phenpip)$^{2+}$ and alanine)

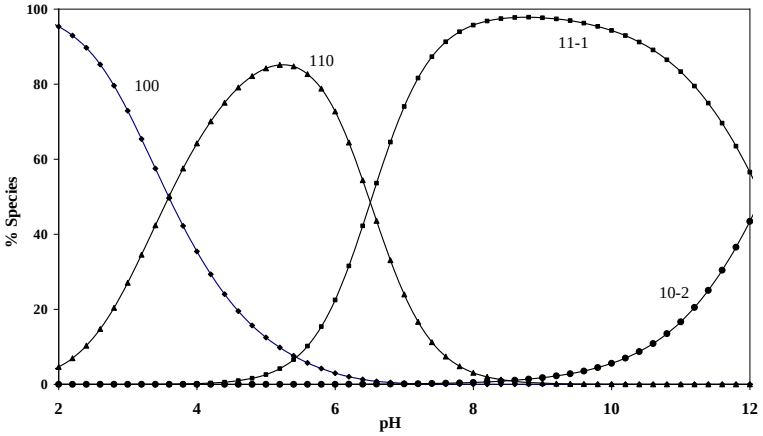

Figure 3. Concentration distribution of various species as a function of pH in the Pd(Phenpip)-glycylglycine system (at concentration of 1.25m ML^{-1} for Pd(Phenpip)$^{2+}$ and glycylglycine)

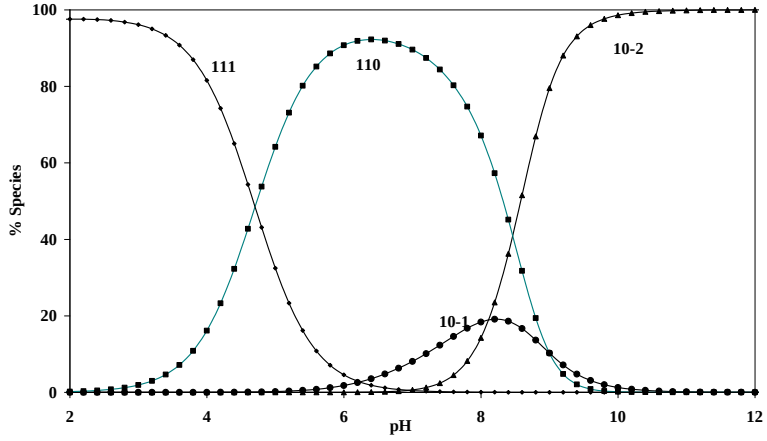

Figure 4. Concentration distribution of various species as a function of pH in the Pd(Phenpip)-oxalic acid system (at concentration of 1.25mML^{-1} for Pd(Phenpip)$^{2+}$ and oxalic acid).Fig 3