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# Complex formation reactions of palladium(II)-1,3-diaminopropane with various biologically relevant ligands. Kinetics of hydrolysis of glycine methyl ester through complex formation

**Research Article**

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**Abstract:** The interaction of $[Pd(DAP)(H_2O)_2]^{2+}$ (DAP = 1,3-diaminopropane) with some selected bio-relevant ligands, containing different functional groups, were investigated. The ligands used are dicarboxylic acids, amino acids, peptides and DNA constituents. Stoichiometry and stability constants of the complexes formed are reported at 25°C and 0.1 M ionic strength. 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 is examined. Peptides form both 1:1 complexes and the corresponding deprotonated amide species. DNA constituents form 1:1 and 1:2 complexes. The effect of dioxane on the acid dissociation constants of CBDCA and the formation constant of its complex with $Pd(DAP)^{2+}$ was reported. The kinetics of hydrolysis of glycine methyl ester bound to $[Pd(DAP)(H_2O)_2]^{2+}$ was studied at 25°C and 0.1M ionic strength.

**Keywords:** *Amino acids • 1,3-diaminopropane • Peptides • DNA constituents and potentiometric titration*



## 1. Introduction

*Cis*-$Pt(NH_3)_2Cl_2$ (cisplatin) is one of the most effective anti tumor agents [1,2]. However, there are still difficulties related to its use, because of numerous side effects and its toxicity. Several methods have been developed during the past ten years which have considerably reduced these side effects. *Cis*-platin is not easily soluble in water and tends to hydrolyze at neutral solution. Replacement of chloro ligands by carboxylate groups in carboplatin [3], leading to *cis*-diamine(1,1-cyclobutanedicarboxylate)-platinum(II) reduces the toxicity of the complex and increases its solubility in water. Some investigators have suggested that carboplatin is merely a pro-drug for cisplatin [4], whereas others have postulated a ring opening reaction [5] of carboplatin followed by the reaction with guanosine 5′-monophosphate (5'-GMP) to form $[Pt(NH_3)_2(CBDCA\text{-}O)(5'\text{-}GMP)]$. The ring opening reaction with DNA constituents was investigated kinetically in a study of the reaction of [Pd(amine)(CBDCA)] with inosine 5′-monophosphate [6]. In order to avoid the inert substitution behaviour 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 to obtain a reasonable picture of the thermodynamics of the reactions for closely related Pt(II) complexes.

Work in our laboratories focused on the studies of metal complexes of biological significance [7-12]. Pd(II) complexes with bidentate amine forming five-membered chelate rings were extensively investigated as a model of the antitumour cis-diamine Pt(II) complex. It seemed of interest to study the behaviour of palladium(II) complexes with 1,3-diaminopropane, involving a six-membered chelate ring. This may be the first step in the development of a new generation of anti tumor agents. Also, the increase in the chelate ring size will increase the bite angle, which increases 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 [13,14]. Such restriction may stabilize the DNA

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adduct. The is line with 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 [15]. For these reasons, it seems therefore of considerable interest to perform a systematic study of the complex formation equilibria between $[Pd(DAP)(H_2O)_2]^{2+}$ and amino acids, peptides, dicarboxylic acids or DNA constituents. Also, the hydrolysis of α-amino acid esters through complex formation with $[Pd(DAP)(H_2O)_2]^{2+}$ was also investigated in aqueous solution at 25°C.

# 2. Experimental Procedure

## 2.1. Materials

$PdCl_2$ and 1,3-diaminopropane (DAP) were obtained from Aldrich Chem. Co. Amino acids, peptides, DNA constitutents and dicarboxylic acids investigated are: glycine, alanine, threonine, glutamic acid, methionine, S-methylcysteine, methylamine.HCl, glycinamide, glycylglycine, glutamine, uracil, thymine, inosine, 1,1-cyclobutanedicarboxylic acid ($CBDCAH_2$), oxalic acid, succinic acid, malonic acid and glycine methyl ester HCl. These materials were provided by Sigma Chemicals company and used without further purification. For equilibrium studies, $[Pd(DAP)Cl_2]$ was converted into the diaqua complex $[Pd(DAP)(H_2O)_2](NO_3)_2$ by stirring the chloro- complex with two equivalents of $AgNO_3$ overnight, and removing the AgCl precipitate by filtration through a 0.1 μm pore membrane filter. Great care was taken to ensure that the resulting solution was free of $Ag^+$ ions and that the chloro- complex had been converted into the aqua species, the filtrate made up to the desired volume in a standard volumetric flask. The ligands in the form of hydrochlorides were converted to the corresponding hydronitrate in the same way as described above. 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 of $[Pd(DAP)Cl_2]$ complex

$[Pd(DAP)Cl_2]$ complex is prepared by heating $PdCl_2$ (0.886 g; 5 mmol) and KCl (0.745 g; 10 mmol) in the least amount of water to 70°C for 30 minutes with stirring. The clear solution of $[PdCl_4]^{-2}$ solution is cooled to 25°C, filtered and 1,3-diaminopropane (0.925 g; 5 mmol), is added dropwise to the stirred solution. A yellow precipitate of $[Pd(DAP)Cl_2]$ is 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 diethylether. 80% yield of yellow powder was obtained. Anal. Calcd. for $C_3H_{10}N_2PdCl_2$, 14.3; H, 4.0; N, 11.1; Cl, 28.2; Found: C, 14.4; H, 4.0; N, 11.1; Cl, 28.1%).

$[Pd(DAP)Cl_2]$ is converted into the diaqua complex $[Pd(DAP)(H_2O)_2](NO_3)_2$ by stirring the chloro complex with two equivalents of $AgNO_3$ overnight, and removing the AgCl precipitate by filteration through a 0.1 μm pore membrane filter. Great care was taken to ensure that the resulting solution was free of $Ag^+$ ions and that the chloro complex had been converted into the aqua species, the filtrate made up to the desired volume in a standard volumetric flask.

## 2.3. Apparatus

Potentiometric measurements were made using a Metrohm 686 titroprocessor equipped with a 665 Dosimat (Switzerland-Herisau). A thermostatted glass-cell was used equipped with a magnetic stirring system, a Metrohm glass-calomel combined electrode, a thermometric probe and a microburet delivery tube. The titroprocessor and electrode were calibrated daily with standard buffer solutions prepared according to NBS specifications at 25.0 ± 0.1°C [16] and I = 0.1 mol $dm^{-3}$, potassium hydrogen phthalate (pH 4.008) and a mixture of $KH_2PO_4$ and $Na_2HPO_4$ (pH 6.865). Elemental microanalysis 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 data.

## 2.4. Procedure and measuring techniques

The acid dissociation constants of the ligands were determined potentiometrically by titrating 40 $cm^3$ of ligand solution ($1.25\times10^{-3}$ mol $dm^{-3}$). The acid-dissociation constants of the coordinated water molecules in $[Pd(DAP)(H_2O)_2)]^{2+}$ were determined by titrating ($1.25\times10^{-3}$ mol $dm^{-3}$) solution of the complex. The formation constants of the complexes were determined by titrating solution mixtures of $[Pd(DAP)(H_2O)_2]^{2+}$ ($1.25\times10^{-3}$ mol $dm^{-3}$) and the ligand in concentration ratios of 1:1 for dicarboxylic acids, amino acids and peptides, and for concentration ratios of 1:1 and 1:2 (metal:ligand) for DNA constituents. The titration solution mixtures had a volume of 40 mL. Temperature was maintained constant inside the cell at (25.0 ± 0.1) °C, by circulating thermostated water through the double-wall titration vessel and under a slow and constant stream of $N_2$ over the test solutions. 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°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 [17]. The ionic strength was adjusted to 0.1 mol $dm^{-3}$ by using $NaNO_3$. The equilibrium constants evaluated from the titration

data (summarized in Table 1) are defined by Eqs. 1 and 2, where M, L and H stand for the $[Pd(DAP)(H_2O)_2]^{2+}$ ion, ligand and proton, respectively.

$$pM + qL + rH \rightleftharpoons M_pL_qH_r \quad (1)$$

$$\beta_{pqr} = \frac{[M_pL_qH_r]}{[M]^p\,[L]^q\,[H]^r} \quad (2)$$

The calculations were obtained from *ca*. 100 data points in each titration using the computer program MINIQUAD-75 [18]. 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, as described elsewhere [18]. The fitted model was tested by comparing the experimental titration data points and the theoretical curve calculated from the values of the acid dissociation constant of the ligand and the formation constants of the corresponding complexes. The results are summarized in Table 1. The species distribution diagrams were obtained using the program SPECIES [19] under the experimental condition employed.

The kinetics of the hydrolysis of the complexed ester were monitored by the pH-stat technique [20,21] by using a titroprocessor operated in the SET mode. The hydrolysis was investigated using an aqueous solution (40 mL) containing a mixture of $[Pd(DAP)(H_2O)_2]^{2+}$ ($1.25\times10^{-3}$) and glycine methyl ester ($1.25\times10^{-3}$) and the ionic strength was adjusted to 0.1 M with $NaNO_3$. The pH of the mixture was progressively raised to the desired value. The reaction was monitored by the addition of NaOH solution to maintain the given pH. The data fitting was performed with the OLIS KINFIT set of programs [22] as described previously [8].

## 3. Results and Discussion

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].

### 3.1. Hydrolysis of $[Pd(DAP)(H_2O)_2]^{2+}$

The hydrolysis of the $[Pd(DAP)(H_2O)_2]^{2+}$ 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.

$$\underset{100}{[Pd(DAP)(H_2O)_2]^{2+}} \rightleftharpoons \underset{10\text{-}1}{[Pd(DAP)(H_2O)(OH)]^{+}} + H^{+} \quad (3a)$$

$$\underset{10\text{-}1}{[Pd(DAP)(H_2O)(OH)]^{+}} \rightleftharpoons \underset{10\text{-}2}{[Pd(DAP)(OH)_2]} + H^{+} \quad (3b)$$

The $pK_{a1}$ and $pK_{a2}$ values were found to be 5.69 and 9.22, respectively. The $pK_{a1}$ value is higher than that for $[Pd(AMBI)(H_2O)_2]^{2+}$ ($pK_a$ 4.7) [10] (AMBI = 2-aminomethylbenzimidazole). This shows that the first coordinated water molecule in $[Pd(DAP)(H_2O)_2]^{2+}$ 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 distribution diagram for $[Pd(DAP)(H_2O)_2]^{2+}$ and its hydrolysed species is shown in Fig. 1. The concentration of the monohydroxo species (10-1) increases with increasing pH, predominating at pH 7, with formation percentage of ca. 96%. Therefore, the main species present in solution in the physiological pH range which can interact with DNA subunits. A further increase in pH is accompanied by an increase in the dihydroxo species, which is the main species above a pH 10. This indicates that, in the high pH range the inert dihydroxo complex would be the predominant species, so that, the reactivity of DNA to bind the Pd(amine) complex will considerably decrease in the high pH range. It has been stated [24] that at a concentration higher than 0.2 mmol $dm^{-3}$ , the dihydroxy bridged dimer is present. The monomer becomes the major species in solution by lowering the total complex concentration. Trials were made to fit the potentiometric data of the present study assuming the formation of the hydroxo-bridged dimer, but this resulted in a very poor fit to the data.

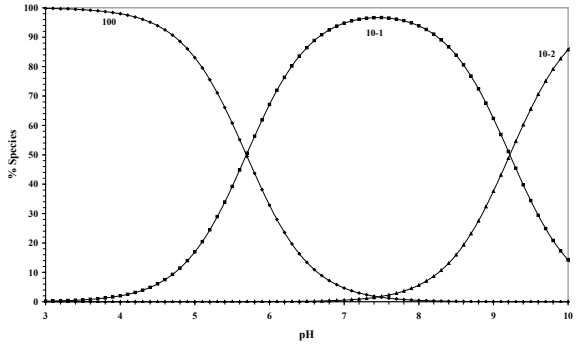



**Figure 1.** Concentration distribution of various species as a function of pH in the hydrolysis of $[Pd(DAP)(H_2O)_2]^{2+}$ at concentration of $1.25\times10^{-3}$ mol $dm^{-3}$, I = 0.1 mol $dm^{-3}$ ($NaNO_3$) and T = 25±0.1°C).

## 3.2. Complex-Formation equilibria involving dicarboxylic acids

In the Pd(DAP)-dicarboxylic acid system, the computer analysis of the pH titration data showed the formation of the 1:1 species. The results (Table 1) show that the formation constant of the 1:1 complex involving formation of five and six membered chelate rings as in cyclobutanedicarboxylic acid, oxalic acid and malonic acid are higher than those involving seven-membered chelate ring, as in succinic acid. This may be explained on the premise that the five-and six-membered rings are more favoured energetically than the seven-membered ring. It is interesting to note that CBDCA has a higher stability constant than malonic acid, although both of them form six-membered chelate ring. This may be due to the higher $pK_a$ values of the former than the latter dicarboxylic acid. The distribution diagram of $[Pd(DAP)]^{2+}$ - CBDCA complex was given in Fig. 2. The main species in the physiological pH range is the ring-closed form, 110, which reaches a maximum concentration of 98.5% at pH ca. 5.5.

## 3.3. Complex formation equilibria involving amino acids

Analysis of pH titration data for Pd(DAP)-amino acid systems showed the formation of 1:1 complexes with stability constants larger than for the corresponding monodentate methylamine complex. This indicates 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 [25] to participate in complex formation. The $pK_a$ value of the alcoholate group incorporated in the Pd(II) complex ($\log \beta_{110} - \log \beta_{11\text{-}1}$) is 8.46. Therefore in physiological pH (7.4), the –OH group participates in bonding with $Pd(DAP)^{2+}$ ion.

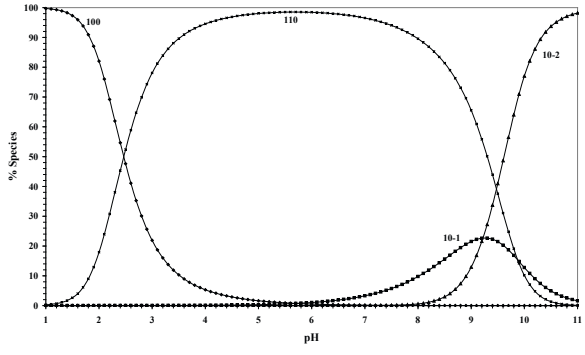



**Figure 2.** Concentration distribution of various species as a function of pH in the Pd(DAP)-cyclobutane dicarboxylic acid (CBDCA) system at concentrations of $1.25\times10^{-3}$ mol $dm^{-3}$ for $Pd(DAP)^{2+}$ and CBDCA, I = 0.1 mol $dm^{-3}$ ($NaNO_3$) and T = 25±0.1°C).

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

| System | p | q | r[a] | logß[b] |
|---|---|---|---|---|
| $Pd(DAP)(OH_2)_2$ | 1 | 0 | -1 | -5.96 (0.02) |
| | 1 | 0 | -2 | -14.91(0.03) |
| 1,1`-Cyclobutanedicarboxylic acid | 0 | 1 | 1 | 5.68 (0.01) |
| | 0 | 1 | 2 | 8.80 (0.01) |
| | 1 | 1 | 0 | 7.16 (0.06) |
| Oxalic acid | 0 | 1 | 1 | 3.93 (0.01) |
| | 0 | 1 | 2 | 5.66 (0.01) |
| | 1 | 1 | 0 | 6.57(0.02) |
| Malonic acid | 0 | 1 | 1 | 5.21 (0.01) |
| | 0 | 1 | 2 | 7.61 (0.01) |
| | 1 | 1 | 0 | 5.63(0.06) |
| Succinic acid | 0 | 1 | 1 | 5.25 (0.01) |
| | 0 | 1 | 2 | 9.27 (0.01) |
| | 1 | 1 | 0 | 4.95(0.05) |
| Glycine | 0 | 1 | 1 | 9.60 (0.01) |
| | 0 | 1 | 2 | 11.93(0.03) |
| | 1 | 1 | 0 | 11.12 (0.02) |
| Alanine | 0 | 1 | 1 | 9.69 (0.01) |
| | 0 | 1 | 2 | 11.88(0.02) |
| | 1 | 1 | 0 | 11.22(0.04) |
| Methionine | 0 | 1 | 1 | 9.10 (0.01) |
| | 0 | 1 | 2 | 11.08(0.03) |
| | 1 | 1 | 0 | 10.31(0.06) |
| Glutamic Acid | 0 | 1 | 1 | 9.42 (0.01) |
| | 0 | 1 | 2 | 13.50(0.02) |
| | 1 | 1 | 0 | 9.72(0.03) |
| | 1 | 1 | 1 | 13.60(0.02) |
| Threonine | 0 | 1 | 1 | 9.06 (0.01) |
| | 0 | 1 | 2 | 11.03(0.02) |
| | 1 | 1 | 0 | 10.57(0.06) |
| | 1 | 1 | -1 | 2.11 (0.06) |
| S-methylcysteine | 0 | 1 | 1 | 8.51(0.01) |
| | 1 | 1 | 0 | 10.64(0.05) |
| Methylamine.HCl | 0 | 1 | 1 | 10.03(0.04) |
| | 1 | 1 | 0 | 7.91(0.07) |
| | 1 | 2 | 0 | 14.63(0.09) |
| Glycinamide | 0 | 1 | 1 | 7.88(0.01) |
| | 1 | 1 | 0 | 8.63(0.08) |
| | 1 | 1 | -1 | 5.23(0.09) |
| Glycylglycine | 0 | 1 | 1 | 7.97(0.01) |
| | 1 | 1 | 0 | 8.01(0.06) |
| | 1 | 1 | -1 | 4.24(0.06) |
| Glutamine | 0 | 1 | 1 | 8.92 (0.01) |
| | 1 | 1 | 0 | 9.24(0.05) |
| | 1 | 1 | -1 | -0.32 (0.08) |
| Uracil | 0 | 1 | 1 | 9.28 (0.01) |
| | 1 | 1 | 0 | 8.61(0.02) |
| | 1 | 2 | 0 | 14.75(0.04) |
| Thymine | 0 | 1 | 1 | 9.58(0.01) |
| | 1 | 1 | 0 | 9.02(0.05) |
| | 1 | 2 | 0 | 15.65(0.03) |
| Inosine | 0 | 1 | 1 | 8.43(0.01) |
| | 1 | 1 | 0 | 7.62(0.04) |
| | 1 | 1 | 1 | 9.69(0.08) |

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

S-methylcysteine forms a more stable complex than methionine, plausibly due to the fact that the five-membered chelate ring in the former complex is energetically more favoured than six-membered chelate ring in the latter complex.

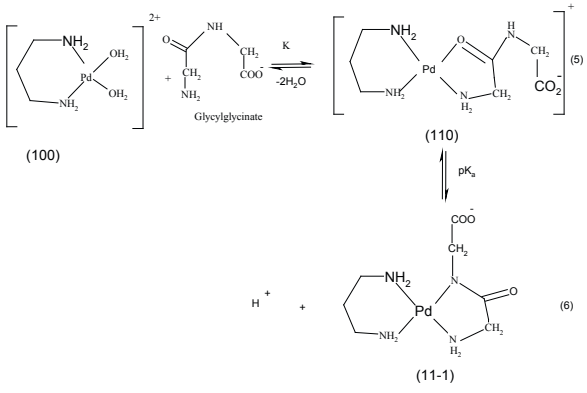
Glutamic acid has two carboxylic and one amino group as potential chelating centres. It may coordinate either by the two carboxylate groups or by the one amino and one carboxylate group. The stability constant of the glutamic acid complex is (larger or smaller than) that of dicarboxylic acids. This may reveal that glutamic acid coordinates by the one amino and one carboxylate group. The acid dissociation constant of the protonated species is given by the following Eq. 4.

$$pK^H = \log \beta_{111} - \log \beta_{110} \quad (4)$$

The $pK_a$ of the protonated species of Pd(DAP)-glutamic acid complex amounts to 3.88, being lower than that of the protonated amino group $-NH_3^+$ ($pK_a$ = 9.42), but closer to that of the protonated carboxylate group ($pK_a$ = 4.08), suggesting the proton in the protonated complex would be located mainly on the carboxylate group.

## 3.4. Complex formation equilibria involving peptides

The potentiometric data for the Pd(DAP)-peptide system 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. 5 and 6.




In the 110 species, the peptide is bound through the amino and carbonyl groups. Upon deprotonation of the amide group, the coordination sites could switch from the carbonyl oxygen to the amide nitrogen such that the 11-1 complex is formed. Such changes in coordination modes are well documented [26]. The glutamine complex is more stable than the glycinamide complex, presumably due to the fact that glutamine carries a negative charge, whereas glycinamide is neutral. The electrostatic interaction between the glutaminate and the positively charged Pd(II) complex would result in the lowering of the free energy of complex formation. The $pK_a$ values of the amide group, incorporated in the Pd(II) complex ($\log\beta_{110} - \log\beta_{11-1}$) are in the range of 3.40-9.56. The low $pK_a$ values in the present study are probably due to the high affinity of Pd(II) to nitrogen donor ligands. It is interesting to note that the $pK_a$ value for the glycinamide complex is lower than that of other peptides. This can be explained on the basis that the more bulky substituent on the peptide may hinder structural changes when going from the protonated to the deprotonated complex. The $pK_a$ of the glutamine complex is exceptionally higher than those of the other peptide complexes. This is due to the formation of a seven-membered chelate ring which is more strained and therefore less favoured. The distribution diagram for the Pd(DAP)-glycylglycine system is given in Fig. 3. $[Pd(DAP)L]^+$ (110) starts to form at lower pH. Its concentration increases with increasing pH and reaches a maximum of 44.8% at pH 3.4. A further increase in pH is accompanied by a decrease in $[Pd(DAP)L]^+$ (110) concentration and an increase in [Pd(DAP)LH-1] (11-1) concentration, reaching a maximum of 99.8% at pH 6.8, *i.e.* in the physiological pH range the deprotonated species (11-1) predominates.

## 3.5. Complex formation equilibria involving DNA constituents

The pyrimidines uracil and thymine have only basic nitrogen donor atoms ($N_3$-$C_4O$ group). They form 1:1 and 1:2 complexes with $[Pd(DAP)(H_2O)_2]^{2+}$. Inosine is slightly more acidic than the pyrimidine bases, a property which can be related to the existence of a higher number of resonance forms for the inosine anion. Based on the existing data, uracil and thymine bind in the deprotonated form through the $N_3$ atom. The thymine complex is more

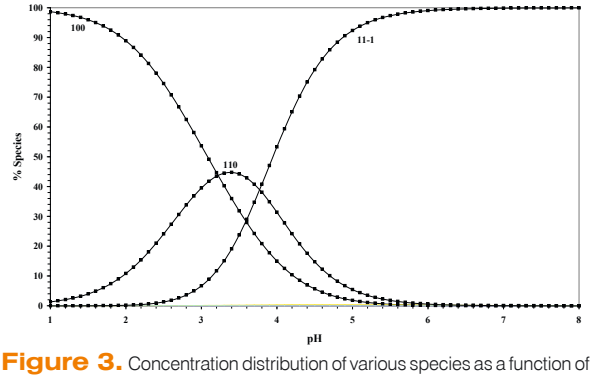



**Figure 3.** Concentration distribution of various species as a function of pH in the Pd(DAP)-glycylglycine system at concentrations of $1.25\times10^{-3}$ mol $dm^{-3}$ for $Pd(DAP)^{2+}$ and glycylglycine, I = 0.1 mol $dm^{-3}$ ($NaNO_3$) and T = 25±0.1°C).

stable than that of uracil, most probably owing to the high basicity of the $N_3$ group of thymine resulting from the extra electron donating methyl group. As a result of the high $pK_a$ values of pyrimidines ($pK_a \approx 9$) and the fact that they are monodentate, the complexes are formed only above pH 6, supporting the view that the negatively charged nitrogen donors of pyrimidine bases are important binding sites in the neutral and slightly basic solution. The purines like inosine have two metal ion binding centres $N_1$ and $N_7$ nitrogens. The pH dependent binding of these N-donors was already reported [27]. The results showed that inosine forms the complexes 110 and 111. The speciation of inosine complex is presented in Fig. 4, where the species distribution of the complexes is plotted as a function of pH. The species 111 is formed in acidic solution and it corresponds to the $N_7$ coordinated complex, while $N_1$ nitrogen is in its protonated form. The $pK_a$ of the protonated form ($\log \beta_{111} - \log \beta_{110}$) amounts to 2.07.

## 3.6. Effect of solvent

Traditionally, water has been considered as the solvent that best represents biological conditions. Although this is a general assumption, a lower polarity has been detected in some biochemical micro-environments, such as active sites of enzymes and side chains in proteins [28-32]. It was suggested that these properties approximately correspond to those (or can be simulated by those) existing in the water/dioxane mixtures. Consequently, a study of the Pd(DAP)-CBDCA complex formation, taken as a typical example, in dioxane-water solutions of different compositions could be of biological significance. In order to characterize the formation equilibria of the Pd(DAP)-CBDCA complex in dioxane-water solutions, all other equilibria involved, viz. acid-base equilibria of CBDCA and $[Pd(DAP)(H_2O)_2]^{2+}$, have to be studied in the same solvent. The equilibrium constants are reported in Table 2. The hydrolysis of $Pd(DAP)^{2+}$ complex in dioxane-water solution leads to the formation of mono- and dihydroxy species. The dihydroxo bridged dimer was not detected. The $pK_a$ values of CBDCA and those of the coordinated water molecules in $[Pd(DAP)(H_2O)_2]^{2+}$ increase linearly with increasing dioxane concentration. This may be correlated with the ability of a solvent of relatively low dielectric constant to increase the electrostatic attraction between the proton and ligand anion in case of CBDCA and between a proton and the hydrolysed form of Pd(II) species. The variation in stability constant of the $[Pd(DAP)(H_2O)_2]^{2+}$ complex with CBDCA as a function of solvent composition with a range from 12.5 to 62.5% is investigated. The stability constant for the Pd(DAP)-CBDCA complex increases with increasing dioxane concentration. This is explained in terms of

**Table 2.** Effect of dioxane on the formation constant of Pd(DAP)-CBDCA complex at 25°C

| %Dioxane | p | q | r[a] | log ß[b] |
|---|---|---|---|---|
| 12.5 % | 0 | 1 | 1 | 6.16(0.01) |
| | 0 | 1 | 2 | 9.58(0.02) |
| | 1 | 1 | 0 | 8.13(0.05) |
| | 1 | 1 | -1 | -5.78(0.03) |
| | 1 | 1 | -2 | -14.99(0.06) |
| 25 % | 0 | 1 | 1 | 6.57(0.01) |
| | 0 | 1 | 2 | 10.38(0.02) |
| | 1 | 1 | 0 | 8.57(0.02) |
| | 1 | 1 | -1 | -5.82(0.04) |
| | 1 | 1 | -2 | -15.21(0.08) |
| 37.5 % | 0 | 1 | 1 | 7.06(0.01) |
| | 0 | 1 | 2 | 11.28(0.01) |
| | 1 | 1 | 0 | 8.98(0.04) |
| | 1 | 1 | -1 | -5.89(0.06) |
| | 1 | 1 | -2 | 15.49(0.09) |
| 50 % | 0 | 1 | 1 | 7.63(0.03) |
| | 0 | 1 | 2 | 12.31(0.03) |
| | 1 | 1 | 0 | 9.48(0.05) |
| | 1 | 1 | -1 | -5.96(0.08) |
| | 1 | 1 | -2 | -15.71(0.09) |
| 62.5 % | 0 | 1 | 1 | 8.09(0.05) |
| | 0 | 1 | 2 | 13.07(0.05) |
| | 1 | 1 | 0 | 10.13(0.07) |
| | 1 | 1 | -1 | -6.05(0.05) |
| | 1 | 1 | -2 | -15.93(0.07) |

[a]p,q and r are the stoichiometric coefficients corresponding to Pd(DAP), CBDCA and $H^+$ respectively.

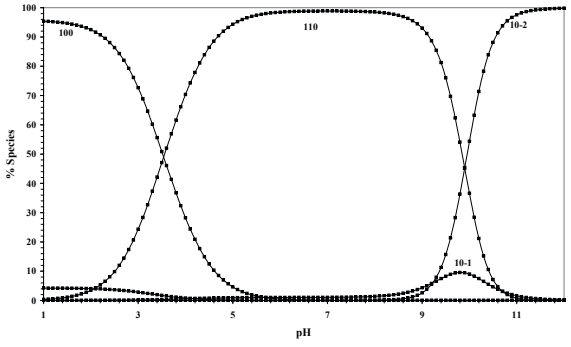



**Figure 4.** Concentration distribution of various species as a function of pH in the Pd(DAP)-inosine system at concentrations of $1.25\times10^{-3}$ mod$m^{-3}$ for $Pd(DAP)^{2+}$ and inosine, I = 0.1 mol$dm^{-3}$ ($NaNO_3$) and T = 25±0.1°C).

complex formation involving oppositely charged ions as in the Pd(DAP)-CBDCA complex, which is favoured by the low dielectric constant of the medium, *i.e.* with increasing dioxane concentration. The results show that the CBDCA complex with $Pd(DAP)^{2+}$ will be more favoured in biological environments of lower dielectric constant.

## 3.7.Kinetics of hydrolysis of amino acid esters

The hydrolysis of the coordinated ester was monitored over the pH range 4.4-5.2. Is there a reference for the following claim? In this range, the rate of hydrolysis of GlyOMe is negligible [20,21] in the absence of the

$Pd(DAP)^{2+}$. The kinetic data, the volume of base added to keep the pH constant versus time, could be fitted by one exponential as shown in Fig. 5. Various other kinetic models were tested but did not produce satisfying fits to the data. The values of $k_{obs.}$ at different $pH_{(s)}$ are given in Table 3. Plot of $k_{obs.}$ versus the hydroxide ion concentration is linear, Fig. 6. The rate expression can therefore be given in the form of Eq. 7

$$k_{obs.} = k_o + k_{OH}[OH^-] \quad (7)$$

the $k_o$ term arises from the attack of water being expressed by the relation 8 [33].

$$k_{H2O} = k_o / 55.5 \quad (8)$$

where 55.5 mol $dm^{-3}$ is the molar concentration of water, $k_o$ presents the rate constant for the water-catalyzed pathway and $k_{OH}$ the rate constant for the base-catalyzed pathway. The value of ko was determined from the intercept and value of $k_{OH} = (k_{obs.} - k_o)/[OH^-]$ and was determined from the slope of the respective plot. The $k_o$ $k_{H2O}$ and $k_{OH}$ values are $2.17\times10^{-4}$ $s^{-1}$, $3.95\times10^{-6}$ $M^{-1}$ $s^{-1}$ and $9.43\times10^{4}$ $M^{-1}$ $s^{-1}$ respectively. The $k_{OH}$ for the hydrolysis of free glycine methyl ester is 1.28 $M^{-1}$ $s^{-1}$ [12]. The linear dependence of the rate constant on the $OH^-$ concentration is consistent with direct attack of $OH^-$ ion on the coordinated ester carbonyl group. The catalysis ratio $C = k_{OH}/k_{OH}^{ester}$, where $k_{OH}^{GlyOMe}$ represents the rate constant for the hydrolysis of the free amino acid ester. The catalysis ratio for coordinated glycine methyl ester equals $7.37\times10^{4}$. A catalysis ratio of such high value is consistent with the structural formula (I) for the mixed-ligand complex, in which there is direct interaction between the Pd(II) ion and the ester carbonyl group [34,35]. Attack of water on the $[Pd(DAP)GlyOMe]^+$ complex as determined by $k_{OH}/k_{H2O}$ ratio is some $10^{10}$ slower than that of hydroxide ion. This large difference in nucleophilicities between $OH^-$ and $H_2O$ is also observed for a variety of metal-complexes [35]. The comparative value of $k_{OH}$ [36] at 25°C for the hydrolysis of glycine methyl ester incorporated in $[Pd(Bpy)]^{2+}$ is $2.0\times10^{5}$ $M^{-1}$ $s^{-1}$. The $k_{OH}$ value for $[Pd(DAP)L]^{2+}$ is lower than $[Pd(Bpy)L]^{2+}$ complex. The high reactivity of $[Pd(Bpy)]^{2+}$ complex is due to π-acceptor property of the pyridine ring, leading to an increase of electrophilicity of the metal center.

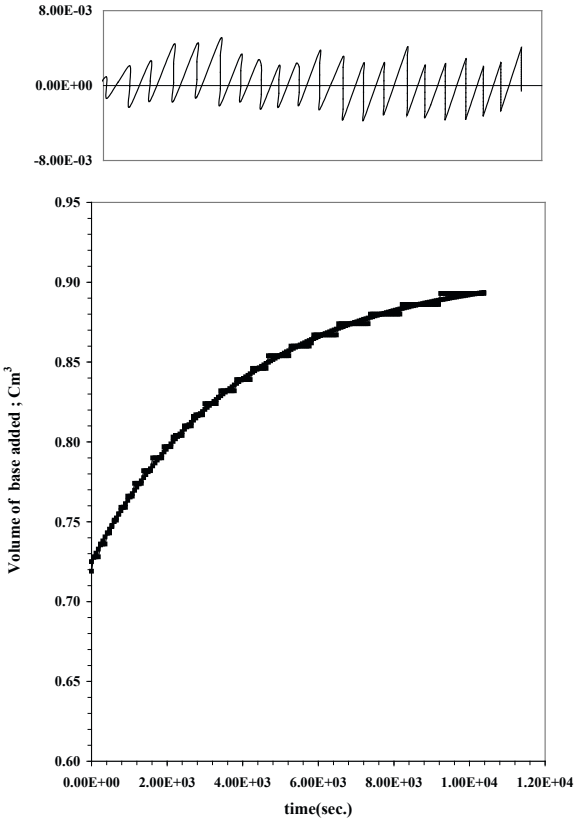



**Figure 5.** Typical volume of base added to keep pH constant-time trace for the hydrolysis of Pd-DAP-Glycine methyl ester fitted with one exponential function. The top of the figure shows the volume of base difference between the measured and calculated kinetic traces at I = 0.1 M; pH = 4.4; T=25°C.

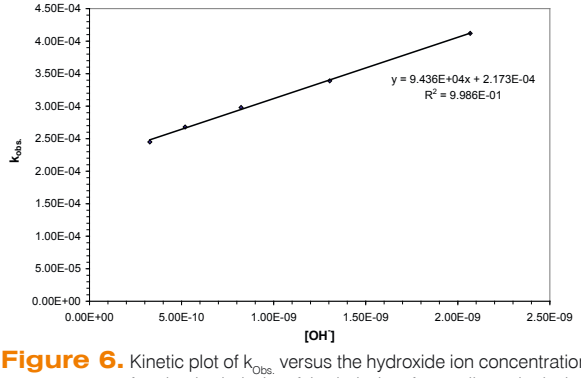
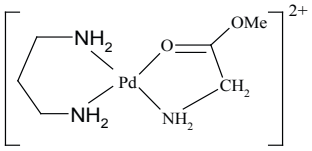



**Figure 6.** Kinetic plot of $k_{Obs.}$ versus the hydroxide ion concentration for the hydrolysis of hydrolysis of coordinated glycine methyl ester at 25°C.

**Table 3.** Kinetics of hydrolysis of the coordinated glycine methyl ester at 25°C and I=0.1 M $NaNO_3$ in aqueous solution

| T °C | pH | $10^{10}$ [OH]$^a$/mol $dm^{-3}$ | $10^4$ $k_{Obs}$/$s^{-1}$ | $10^4$ $k_o$/$s^{-1}$ | $10^6$ $k_{H2O}$/$dm^3$ $mol^{-1}$ $s^{-1}$ | $10^{-4}k_{OH}$/ $dm^3$ $mol^{-1}$ $s^{-1}$ |
|---|---|---|---|---|---|---|
| 25 | 4.40 | 3.28 | 2.45 | 2.17 | 3.91 | 9.43 |
| | 4.60 | 5.19 | 2.68 | | | |
| | 4.80 | 8.23 | 2.98 | | | |
| | 5.00 | 13.0 | 3.39 | | | |
| | 5.20 | 20.7 | 4.12 | | | |

[a] $pK_w$ 13.997 at 25°C and an activity coefficient γ of 0.772.




**Scheme 1.** $[Pd(DAP)(GlyOMe)]^{2+}$

## 4. Conclusions

The present investigation describes the interaction of $[Pd(DAP)(H_2O)_2]^{2+}$ with ligands of biological significance. Through the analysis of stability constants data of the complexes formed with dicarboxylic acids, amino acids, peptides and DNA constitutents, it would be possible to calculate, by using the program SPECIES, the equilibrium distibution of the Pd(II) species in biological fluids where all types of ligands are present simultaneously. This would provide a clear basis for understanding the mode of action of such metal species under physiological conditions. From the above results it may be concluded that CBDCA, among the dicarboxylic acid ligands, forms the highest stable complex, which is inconsistent with the fact that CBDCA complexes has the highest antitumor activity. The stability constants of Pd(picolylamine)$^{2+}$ complexes [9] were compared with those of the present study. The corresponding complexes with picolylamine are more stable than those of 1,3-diaminopropane. This is attributed to the bi acceptor property of the pyridine ring of picolylamine.

Amino acids form the highly stable complexes. The β- alcoholate group in the side chain of the amino acid threonine has been found to play an essential role in the functionating of a number of proteolytic enzymes, *e.g.* chymotrypsin and subtilisin. $[Pd(DAP)(H_2O)_2]^{2+}$ is promoting the ionization of the alcohol group of π threonine. The $pK_a$ of the alcoholate group incorporated in Pd(II) complex is 8.46, which indicates that the participation of the OH group in complex formation contributes to the physiological pH range. The present study clearly shows that the $[Pd(DAP)(H_2O)_2]^{2+}$ complex can form strong bonds with peptides and promotes facile deprotonation of the peptide. A study of the Pd(DAP)-CBDCA complex formation, taken as a typical example, in dioxane-water solutions of different compositions could be of biological significance. The results show that the CBDCA complex with Pd(DAP)$^{2+}$ will be favoured in biological environments of lower dielectric constant. The results of kinetic measurements indicate that the hydrolysis of the glycine methyl ester is significantly catalyzed by the $[Pd(DAP)]^{2+}$ complex.

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