4

Coordination Chemistry of Palladium(II) Ternary Complexes with Relevant Biomolecules

Ahmed A. El-Sherif*
Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt

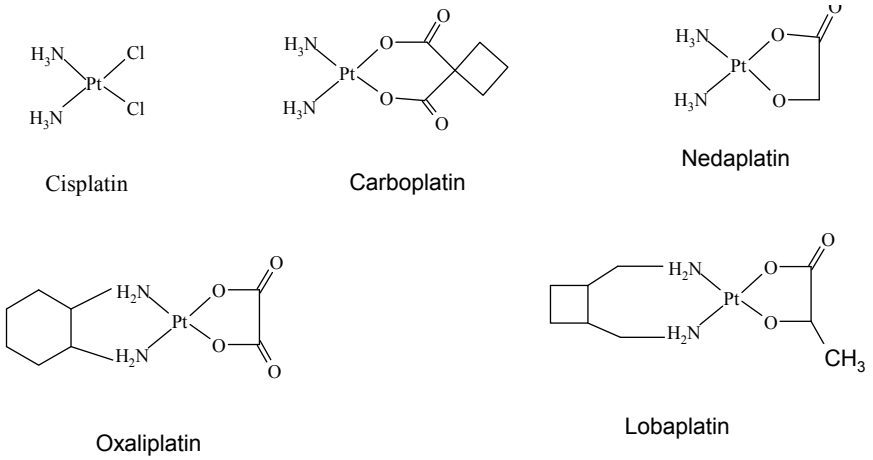
1. Introduction

*Cis*platin, *cis*-Diamminedichloroplatinum (II), is one of the most effective anticancer agents (Rosenberg, 1969). It has demonstrated a remarkable chemotherapeutic potential in a large variety of human solid cancers, such as, testicular, ovarian, bladder, lung and stomach carcinomas (Wong and Giandomenico, 1999; Guo and Sadler, 2000). The successful use of platinum (II) complexes as potent anticancer drugs has attracted the interest of many scientists. It was observed that the nature and arrangement of the ligands can affect the mode of action and metabolism of the drug while crossing the cell membrane and inside the cell. Despite the widespread use of *cis*-platin as an anticancer drug there is still scope for improvement, with respect to: i) reduced toxicity; ii) increased clinical effectiveness; iii) broader spectrum of action; iv) elimination of side effects (e.g., nausea, hearing loss, vomiting, etc); v) increased solubility and vi) ability to use them in combination with other drugs, limited by severe toxicities so far. Replacement of the chloro ligands by carboxylate groups in carboplatin, *cis*-diamine(1,1cyclobutanedicarboxylate)platinum(II), is a widely used second-generation platinum anticancer drug showing less side effects than *cis*-platin. The development of several new anticancer platinum drugs including Carboplatin, Nedaplatin, Lobaplatin and Oxaliplatin (Scheme 1) still have draw-backs and offer no more clinical advantages over the existing cisplatin (Gill, 1984; Galanski et al., 2005; Momekov et al., 2005). Furthermore, the development of acquired resistance to cis-platin is frequently observed during chemotherapy (Heim, 1993).

There is also much interest in Pd(II) analogues because they are usually isostructural with those of Pt(II), which show a very similar coordination process and geometry. However, Pd(II) systems attain equilibrium much more quickly than Pt(II) systems (~10^4-10^5 faster kinetics). The slow formation kinetics for Pt(II) complexes generally rules out the determination of stability constants. Therefore, Pd(II) complexes are frequently used as model complexes to study the interaction of Pt(II) with DNA and to mimic the binding properties of various platinum(II) species (Tercero-Moreno et al., 1996). It was also suggested that the faster aquation of palladium(II) compared with platinum(II) *in vitro*, makes the former a better model for studying Pt(II) reactions *in vivo* (Nelson, et al.,1976) with biological molecules, since these reactions always start with the aquation of the platinum(II) complexes. Several palladium complexes have been reported (Gill, 1984) with

*Corresponding Author

bidentate amine ligands which have shown anticancer activity comparable to or greater than cisplatin. Moreover, a series of labile Pd(II) complexes have proved to be useful as models to obtain a reasonable picture of the thermodynamics of the reactions for closely related Pt(II) complexes. It has also been suggested that these palladium complexes may be useful for the treatment of tumors of the gastrointestinal region where cisplatin fails. Mono-dentate ligands can bind in both cis- and *trans* arrangements around the metal and the isomers stability depend on several factors. Consequently, bidentate ligands are more reliable for the preparation of *cis*-complexes, in particular with palladium(II) and platinum(II) (Misra et al., 1998; Byabartta et al., 2001; Santra et al., 1999; Pal, et al., 2000; Rauth, et al., 2001; Roy et al., 1996; Das, et al., 1997; Das, et al., 1998). The reaction of DNA bases with Pd(II)/Pt(II) complexes of chelating N,N'-donors having *cis*-MCl_2 configuration constitutes a model system which may allow for exploration of the mechanism of the anti-tumor activity of cisplatin. Considering the importance of palladium complexes as potential anticancer drugs, we report here the coordination chemistry of mixed-ligand palladium complexes of bidentate amines with biologically active ligands.



Scheme 1. Platinum-based drugs currently in clinical use.

2. Methods used for detection of aqueous solution complexes

Ion-selective electrodes were used for determination of the position of dynamic equilibrium system and the most common one is the glass electrode or hydrogen gas electrode which can be used for hydrogen ion measurements. Metal-ion selective electrodes or metal-amalgam electrodes can also be used for certain metal ions, but they are seldom as precise or convenient as the hydrogen ion electrode.

A great advantage with the use of ion-selective electrode measurements is that series of data can be easily collected through a titration procedure. From an initial analytical composition, stepwise changes with a burette are made with intervening electrode recordings. The elapsed time between these changes must be certained to be sufficient for equilibrium to be attained. A good method to check for this pre-requisite is to make repeated high-resolution electrode readings at predetermined time intervals, since this will make sluggish attainments of equilibrium clearly visible.

In addition, other experimental techniques are sometimes available. For example, if the metal ion or the ligand is coloured, and the colour changes (in intensity and/or frequency) upon complexation, spectrophotometry can be used. If the metal ion is diamagnetic, or if the ligand contains a suitable nucleus, nuclear magnetic resonance (NMR) is a striking method. This latter method, which ideally gives one separated signal for each unique chemical surrounding, can provide information not only on the free metal ion or ligand concentration, but also on the number of species and their respective concentration for a given analytical composition. Furthermore, since the positions of these signals are susceptible to protonation/deprotonation reactions, they can also be used to gain information on acid/base reactions of ligands and their complexes. Stopped-flow technique can be applied for fast reactions.

2.1 Determination of stability constants of mixed ligand complexes

The solution equilibria between metal ions and ligands may be described by two word-continuous competitions: The proton and a range of metal ions compete for a range of donor sites, the contest being ruled by concentration and pH conditions. The determination of equilibrium constants is an important process for many branches of chemistry (Motekaitis and Martell, 1988) Equilibrium constants can be determined from potentiometric data and/or spectrophotometric data. Developments in the field of computation of equilibrium constants from experimental data were reviewed by Leggett (Legget, 1985) and Meloun et al.[(Meloum, et al., 1994). Since then, many more programs have been published, mainly so as to be able to use microcomputers for the computations. The most commonly used programs for solution equilibrium constant determination are given in Table 1 (Motekaitis and Martell, 1988; Sabatini et al., 1974; Gans et al.,1976; Gans et al.,1985; Zekany and Nagypal, 1985; Sabatini et al., 1992; Gordon, 1982; Chandler et al., 1984; Perrin and Stunzi, 1985 ; Beltrán et al., 1993; Frassineti et al., 1995; Gampp et al.,1985; Tauler, et al., 1991). All of these programs use least-square refinements to reduce the differences between calculated and experimental data to get the best model from the best fit. The sum of square of residuals between experimental and calculated values are normally very small, it is typically between 10^{-6}-10^{-9}. Potentiometry generally used for measurements of formation constants of metal complexes is based on pH-metric titration of the ligand in absence and presence of metal ions. The formation constants derived by the least squares analysis of potentiometric data can describe completely the solution equilibria. The measurements are usually carried out at a constant ionic strength higher than the metal ion concentration. Therefore, no appreciable change in the ionic strength of the solution medium occurs. In general for the reaction:

$$L(M) + p(L_1) + q(L_2) + r(H) \rightleftharpoons [(M)_l(L_1)_p(L_2)_q(H)_r] \quad (1)$$

The overall stability constant, β_{lpqr}, can be calculated from:

$$\beta_{lpqr} = [(M)_l(L_1)_p(L_2)_q(H)_r]/[M]^l[L_1]^p[L_2]^q[H]^r \quad (2)$$

(charges are omitted for simplicity)

where M, L_1, L_2 and H stand for $[Pd(diamine)(H_2O)_2]^{2+}$ ion, ligand(1), ligand(2) and proton, respectively. For OH^- the coefficient (r) for H = -1.

Program	Data type[a]	Reference
PKAS	V	(Motekaitis and Martell, 1988)
MINIQUAD	V	(Sabatini et al., 1974)
MINIQUAD75	V	(Gans et al.,1976)
SUPERQUAD	V	(Gans et al.,1985)
PSEQUAD	V, A	(Zekany and Nagypal, 1985)
HYPERQUAD	V, A	(Sabatini et al., 1992)
TITAN	V	(Gordon, 1982)
SCOGS2a	V	(Chandler et al., 1984)
SCOGS2b	V	(Perrin and Stunzi, 1985)
STAR	A	(Beltrán et al., 1993)
HYPNMR	N	(Frassineti et al., 1995)
SPECFIT	A(E)	(Gampp et al.,1985)
SPFAC	A(E)	(Tauler, et al., 1991)

[a]Additional data types used in calculations: E, ESR and N, NMR

Table 1. The most commonly used programs for calculating equilibrium constants from potentiometric (V) and spectrophotometric (A) data.

2.2 Determination of stability constants of Pd(II) complexes

As mentioned earlier, determination of formation constants of the Pd(II) complexes is made more difficult than in the case of other metals as a result of the unstable nature of $[Pd(H_2O)_4]^{2+}$ in aqueous solution. Some authors have used $[PdCl_4]^{2-}$, as the metal ion source, but in this case the inclusion of the Cl^- as a ligand as well as other ligands in the calculation of formation constants becomes necessary (Bóka, et al., 2001). Elding (Elding, 1972) calculated log β_{14} for $[PdCl_4]^{2-}$ to be ~ 10. Therefore, $[Pd(NH_3)_2(H_2O)_2]^{2+}$ and $[Pd(diamine)(H_2O)_2]^{2+}$ were considered as the starting metal ions for Pd(II) and the acid-base equilibria of the diaquo complexes were first determined. Secondary ligands were then introduced and the formation constant of the mixed ligand complex calculated using one of the above mentioned programs. Hydrolytic reactions of Pt(II) and Pd(II) complexes are important issues because they are related to the action of the *cis*-platinum(II) anti-cancer drugs. The very high thermodynamic stability constants of the chelated diamine complexes of palladium(II) result in the complete formation of the species $[Pd(diamine)(H_2O)_2]^{2+}$ even under very acidic conditions (pH <2), while the relatively high ratios of the stepwise stability constants suppress the bis(bidentatediamine) complex formation in equimolar solution (Nagy and Sóvágó, 2001). As a consequence, all the Pd(II) species are present in the form of $[Pd(bidentatediamine)]^{2+}$, and therefore, the ternary complex, Pd(bidentate diamine)-ligand, can be treated as a binary complex.

2.3 Preparation of $[Pd(diamine)(H_2O)_2]^{2+}$ complex

$[Pd(diamine)Cl_2]$ complexes were prepared, by reaction of $[PdCl_4]^{2-}$ with diamine in the molar ratio 1:1. For equilibrium studies, $[Pd(diamine)Cl_2]$ was converted into the diaqua complex $[Pd(diamine)(H_2O)_2](NO_3)_2$ 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. Also, the ligands in the form of hydrochlorides were converted to the corresponding hydronitrate in the same way as described above.

2.4 Speciation distribution as a function of pH

Speciation (based on concentrations of metal ions and complexing species) refers to a program (Pettit) which calculates and plots the species distribution of a series of complexes over a specified pH range. In this program, the input data of total concentrations of metal and ligand, pH range and the best fit set of β values are used to compute equilibrium concentrations of all the available complex species over the given pH range. All types of complexes can be calculated, including mixed complexes, protonated, hydroxo and polynuclear species. The graphical output can thus provide a visual record of the most predominant complex species at any pH especially within the physiological pH range.

2.5 Determination of the acid-base equilibria of $[Pd(diamine)(H_2O)_2]^{2+}$ complex

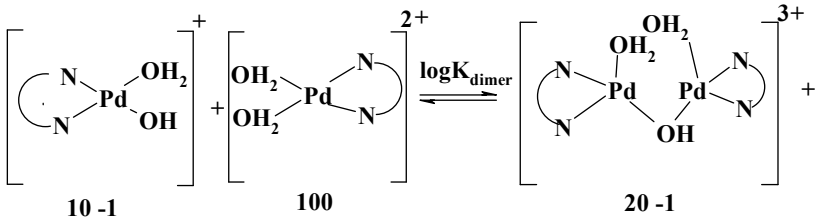
The hydrolysis reactions of Pt(II) complexes are among the most important issues which should be considered under physiological conditions. As a consequence, hydrolysis of cisplatin and its derivatives has been thoroughly studied in both solution and solid state (Martin, 1983; Martin, 1999; Faggiani et al., 1977; Faggiani et al., 1977). It is clear from these studies that hydrolysis of cisplatin and other *cis*-diamine platinum(II) species can not be described by the formation of simple monomeric dihydroxo complexes, but various dinuclear (Faggiani et al., 1978) and trinuclear species are also formed (Faggiani et al., 1977; Faggiani et al., 1977). The very slow formation kinetics, however, hampers the determination of stability constants of platinum(II), but the corresponding palladium(II) complexes can be used as appropriate model compounds (Tercero-Moreno et al., 1996).

The main species formed during the hydrolysis of $[Pd(diamine)(H_2O)_2]^{2+}$ ion are 10-1, 10-2, 20-1 and 20-2. The first two species are due to deprotonation of the two coordinated water molecules, as given by Eqs. 3 and 4.

$$\underset{100}{[Pd(N\text{-}N)(H_2O)_2]^{2+}} \overset{pK_{a1}}{\rightleftharpoons} \underset{10\text{-}1}{[Pd(N\text{-}N)(H_2O)(OH)]^{+}} + H^{+} \quad (3)$$

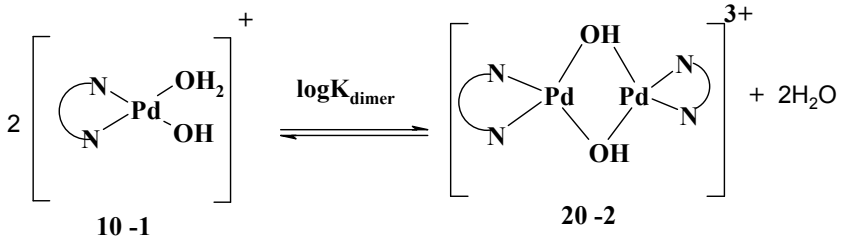
$$\underset{10\text{-}1}{[Pd(N\text{-}N)(H_2O)_2(OH)]^{+}} \overset{pK_{a2}}{\rightleftharpoons} \underset{10\text{-}2}{[Pd(N\text{-}N)(OH)_2]} + H^{+} \quad (4)$$

The third species, (20-1), is the hydroxo bridged-dimer formed as result of the combination of the monoaqua hydroxo species (10-1) with the diaqua species (100) $[Pd(diamine)(H_2O)_2]^{2+}$ as given by Eq. 5.



(5)

The fourth species, 20-2, is the dimeric di-μ-hydroxo complex of two 10-1 species according to Eq.6.



(6)

According to the data in Table 2 (Britten et al., 1982; Lim et al., 1976; Hohmann, et al., 1991; Shoukry, et al., 1999; El-Sherif, et al., 2003 ; Shehata, 2001), the pK_{a1} and pK_{a2} values in the bipyridine as a non-leaving group were found to be 3.91 and 8.39, respectively and are lower than the corresponding values of all the Pd^{II}-diamine complexes. The $[Pd(Pic)(H_2O)_2]^{2+}$ values are intermediate because Pic has one pyridine ring. This can be attributed to the increased positive charge on Pd atom due to the π-acceptor properties of the aromatic moiety of Pyridine ring, leading to an increase in the electrophilicity of the Pd ion and consequently to a decrease in the pK_a of the coordinated water molecule. The equilibrium constant for the dimerization reactions (5) and (6) can be calculated with Eqs. 7 and 8 respectively.

$$\log_{10} K_{dimer} = \log \beta_{20\text{-}1} - \log \beta_{10\text{-}1} \quad (7)$$

$$\log_{10} K_{dimer} = \log \beta_{20\text{-}2} - 2 \log \beta_{10\text{-}1} \quad (8)$$

The concentration distribution diagram for $[Pd(AMBI)(H_2O)_2]^{2+}$ and its hydrolysed species as a representative example of hydrolysis of $[Pd(diamine)(H_2O)_2]$ is shown in Fig.1. The concentration of the monohydroxo species, 10-1 and the dimeric species, 20-2 increase with increasing pH, predominating in the pH range 4.8 to 7.8 with formation percentages of ca. 44% and 54% for the monohydroxo (10-1) and dimeric species (20-2), respectively, i.e., they are the main species present in solution in the physiological pH range. A further increase in pH is accompanied by an increase in the dihydroxo species, which is the main species above a pH of ca. 11. 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.

Complex[a]	Pk_{a1}	pK_{a2}	Reference
Cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$	5.6	7.3	(Britten et al., 1982)
$[Pt(en)(H_2O)_2]^{2+}$	5.8	7.6	(Lim et al., 1976)
$[Pd(en)(H_2O)_2]^{2+}$	5.6	7.3	(Hohmann, et al., 1991)
$[Pd(1,2\text{-}DAP)(H_2O)_2]^{2+}$	5.62	9.35	(Shoukry, et al., 1999)
$[Pd(Pic)(H_2O)_2]^{2+}$	4.81	8.46	(El-Sherif, et al., 2003)
$[Pd(BPY)(H_2O)_2]^{2+}$	3.91	8.39	(Shehata, 2001)

[a]en, 1,2-DAP, Me_2en, Pic and BPY represent ethylenediamine, 1,2-diaminopropane, N,N′-dimethylethylenediamine, picolylamine and 2,2′-bipyridyl, respectively.

Table 2. Comparison of acid dissociation constants of some Pt and Pd-diaquo complexes.

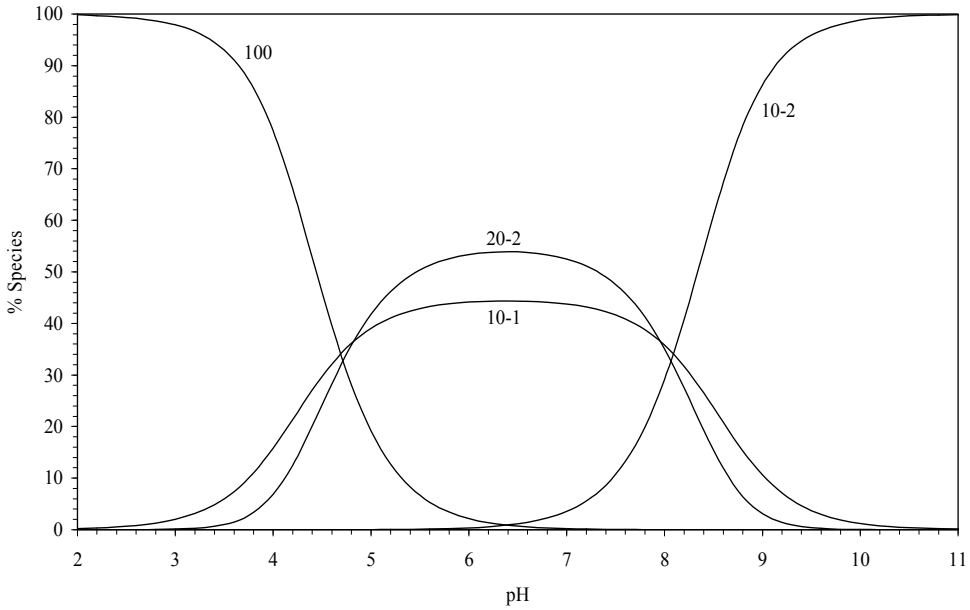


Fig. 1. Concentration distribution of various species as a function of pH in the $Pd(AMBI)(H_2O)_2$ system at concentration of $1.25x10^{-3}$ mol-dm^{-3}, I = 0.1 mol-dm^{-3} ($NaNO_3$) and T = 25 ± 0.1 ^{0}C).

3. Interactions of [Pd(diamine)(H_2O)$_2$] with bio-relevant ligands

3.1 Interactions of [Pd(diamine)(H_2O)$_2$] with amino acids

The potential donor atoms in the amino acids are (the amino-N, the carboxylato- O, as well as the other donor atoms which may be present in the side chains). Pd(II) and Pt(II) form stable complexes with the N-, O-, S-donor atoms present in amino acids, with thermodynamic preference for S- and N- donors over O-donors. Though the thermodynamic preference of the metal ion for a particular donor atom is a very important parameter in determining the choice of donor atoms, at the pH value used for the experiment, these donor atoms may be protonated. Additionally, the effect of chelate ring size may also be a factor in determining the adopted coordination mode.

3.1.1 Acid-base equilibria of amino acids

All amino acids undergo two reversible proton dissociation steps in fairly well separated pH ranges, proceeding according to equilibrium reaction (9).

$$\underset{(H_2L^+)}{{}^+NH_3CH(R)COOH} \overset{pH=2-3}{\rightleftharpoons} \underset{(HL^{\pm})}{{}^+NH_3CH(R)COO^-} \overset{pH=8-10}{\rightleftharpoons} \underset{(L^-)}{NH_2CH(R)COO^-} \text{ ---------- } (9)$$

Besides these two functional groups, most of the essential amino acids contain further functional groups in the side chains of amino acids.

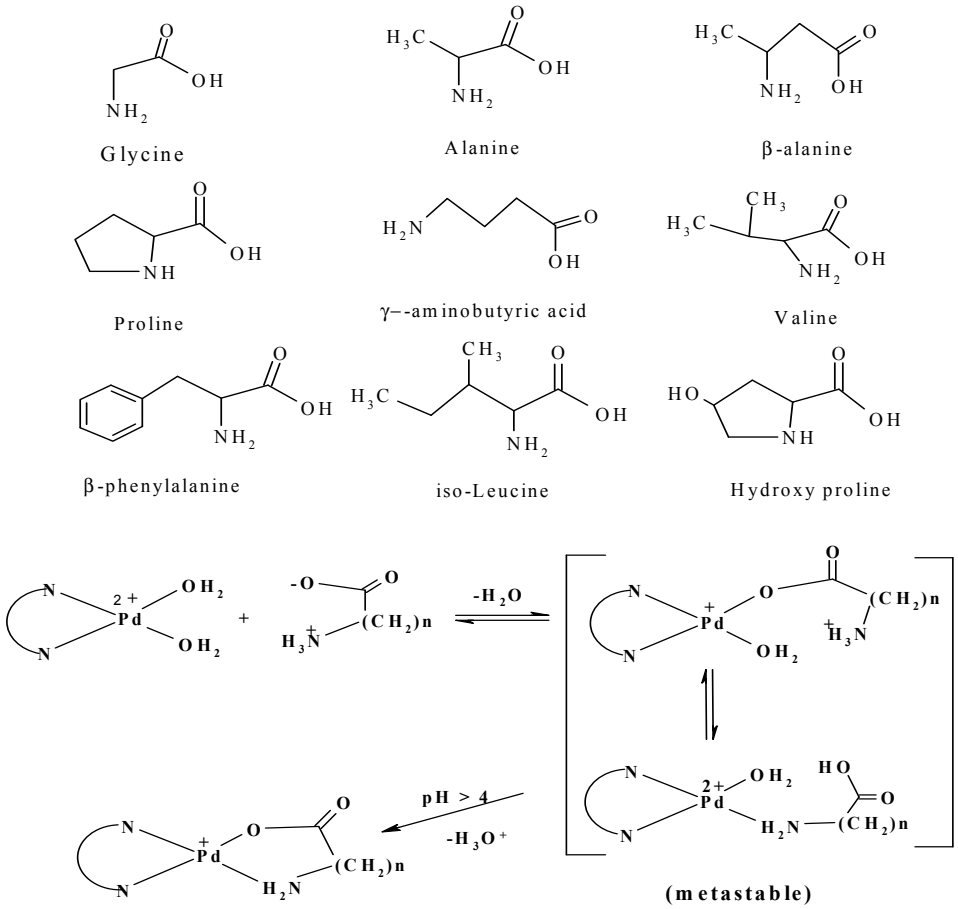
3.1.2 Interactions of [Pd(diamine)(H_2O)$_2$] with amino acids containing no functional group in the side chain

3.1.2.1 Complexes formed when only one metal coordination site is available

Multi-NMR studies (Appleton et al., 1986; Appleton, 1997) of complexes as $[M(NH_3)_3(H_2O)]^{2+}$, M = Pt(II) or Pd(II) with amino acids (AA) showed the initial formation of metastable isomer e.g. $[Pd(NH_3)_3(HGly\text{-}O)]^{2+}$ is formed at low pH~3, which coordinate through the carboxylate oxygen. Since glycine nitrogen (pK_a ~ 9.6) is protonated under these conditions and the carboxyl group (pK_a ~ 2.3) partially deprotonated, carboxylate oxygen is more available for reactions than the amine nitrogen. This complex was slowly converted to $[Pd(NH_3)_3(HGly\text{-}N)]^{2+}$ isomer and the conversion can be slowed at lower pH. β-alanine has an additional methylene group. Its corresponding complex, Hβala-O, did not isomerise at pH = 4.5, but slowly isomerise at higher pH to the complex with β-ala-N. Appleton et al. (Appleton et al., 1986) using multinuclear NMR showed that γ–aminobutyric acid complex, $[Pt(NH_3)_3(\gamma aba\text{-}O)]^{2+}$, standing at pH = 10 caused only slow displacement of the carboxylate-bound ligand by hydroxide. Generally, the O-bound isomer is thermodynamically more stable relative to N-bound form for Pd(II) relative to Pt(II), reflecting a kind of hardness Pd(II) compared to Pt(II) (Appleton, 1997).

3.1.2.2 Complexes formed when two metal coordination sites are available

It has been well established that N,O-chelation is a characteristic coordination mode for glycine bound to palladium(II) (Freeman and Colomb, 1964). The complex-formation equilibria for amino acids may be represented as shown in scheme 2.

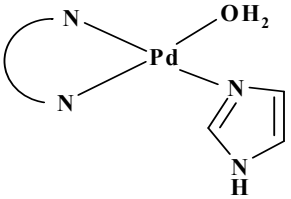


Scheme 2. Coordination mode of amino acids

The complexes of general fomula [Pd(diamine)(AA)], where AA = glycine, alanine, valine, proline, phenylalanine, γ-aminobutyric acid, β-alanine and proline are investigated. The potentiometric titration curves of the [Pd(diamine)] with amino acids, lie significantly below the amino acid alone ones. This reveals that the formation of complex species occurs through release of hydrogen ions.

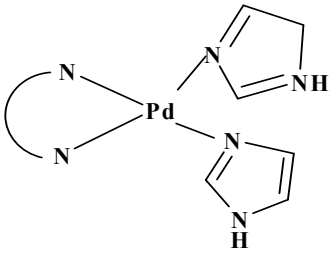
The stability constants of amino acids with [Pd(amine)(H_2O)$_2$] are showed in Tables 3 and 4 (Shoukry et al., 1999; Lim, (1978); Mohamed. and Shoukry, 2001; El-Sherif et al., 2010; Shehata et al., 2008; Shehata et al., 2009; El-Sherif, 2006). The amino acids with no functional groups in the side chains generally form 1:1 complexes with [Pd(diamine)(H_2O)$_2$]$^{2+}$. Their complexes are very stable, with stability constants of log β_{110} ~ 10-12. They coordinate through both the amino group and the carboxylate oxygen, forming stable five-membered chelate ring.

The stability constant of 1:1 complex with imidazole have a smaller value than those of amino acids, further supporting that amino acids are coordinating as bidentate ligands.



$[Pd(N\text{-}N)(imidazole)]^{+2}$

(110)



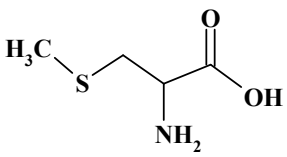
$[Pd(N\text{-}N)(imidazole)_2]^{+2}$

(120)

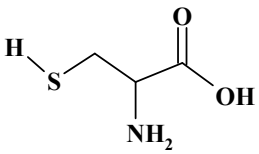
The stability constants log β_{110} of $[Pd(AEPY)alanine]^{2+}$ (10.46) > $[Pd(AEPY)\text{-}\beta\text{-}alanine]^{2+}$ (9.81) > [Pd(AEPY)-γ-aminobutyric acid] (7.81). This trend is attributed to extra stability of five-membered chelate rings for alanine complexes compared to six and seven-membered rings for β-alanine and γ-aminobutyric acid, respectively (Shehata et al., 2009).

3.1.3 Interactions of $[Pd(diamine)(H_2O)_2]$ with amino acids containing sulphur atom in the side chain

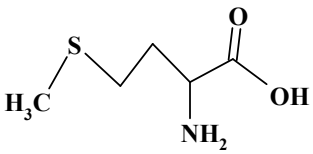
Sulphur containing amino acids (e.g. cysteine, methionine and S-methylcysteine) easily react with Pd(II) because of the great tendency of sulphur (a soft Lewis base) to form bonds with these metals (soft Lewis acids).



S-Methyl cysteine



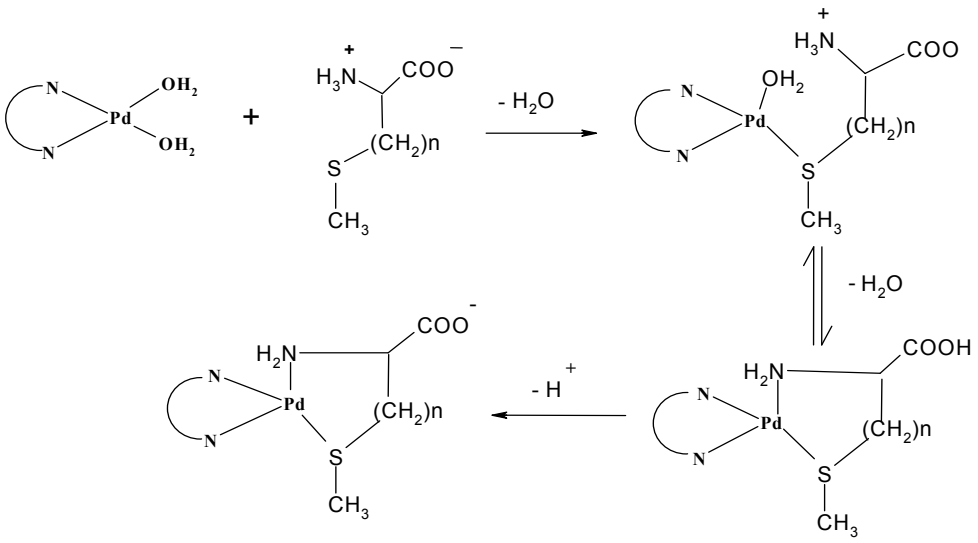
Cysteine



Methionine

The interaction of Pt(II) and Pd(II) with methionine in aqueous solution primarily occurs through the sulphur atom and chelates only in a further step, binding through the amino group (Norman et al., 1992).

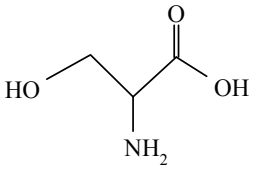
The stability constants of S-methyl cysteine (SMC) and methionine with Pd(II) (tables 3 and 4) are lower than those of ordinary amino acids, suggesting that they are not coordinated as glycine complexes. The stability constants with SMC are generally higher than those with methionine due to the formation of more stable 5-membered ring. The reaction between $[Pd(diamine)(H_2O)_2]^{2+}$ and S-containing amino acids is showed in scheme 3. At low pH values the coordination site is through S-atom, slowly forming bidentate ligand followed by deprotonation of the carboxylic group.



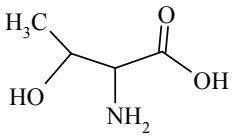
Scheme 3. Interaction of $[Pd(diamine)(H_2O)_2]^{2+}$ with S-containing amino acids.

3.1.4 Interactions of $[Pd(diamine)(H_2O)_2]$ with amino acids containing hydroxy group in the side chain

Serine and threonine are α-amino acids with β-OH group in the side-chain. They contain only two dissociable protons in the measurable pH range ($-NH_3^+$ and -COOH), as the alcoholic hydoxy group is so weakly acidic ($pK_a > 14$), that it does not undergo dissociation in the measurable pH range. The β-alcoholate group in the side chain of the amino acids serine and threonine have been found to play an essential role in the action mechanism of a number of proteolytic enzymes, e.g. chymotrypsin and subtilisin (Bernhard, 1986).



Serine



Threonine

$[Pd(diamine)(H_2O)_2]^{2+}$ promotes the ionization of the alcohol group of serine and threonine, the presence of the species 11-1 indicates the ionization of the OH side-chain. The pK_a of the ionization can be calculated using Eq.10

$$pK_a = \log \beta_{110} - \log \beta_{11-1} \quad (10)$$

The pK_a of ionization for serine and threonine are 8.26 and 7.53 with Pd(1,2-DAP), respectively (Shoukry et al.,1999). Values of 8.51 and 8.05 were obtained with Pd(en), Table 3 (Shoukry, et al., 1999; Lim, 1978; Mohamed and Shoukry, 2001; El-Sherif et al., 2010; Shehata,

et al., 2008). This large acidification of ~ 6 log units indicates a large contribution of the OH group in the coordination process at higher pH and participation of the OH group in complex formation is not contributing significantly in the physiological pH range. The pK_a value of the alcoholate group incorporated in the Pd(II)-AMBI-serine complex is 8.21 (El-Sherif, 2006). This value is lower than that of the Pd(N,N´-dimethylethylenediamine)-serine complex (8.43) (Mohamed and Shoukry, 2001). This may be due the π-acceptor property of the pyridine ring, which increases the electrophilicity of the Pd(II) ion and consequently decreases the pK_a value of the coordinated alcoholate group.

System	p	q	r[b]	en[c]	$(Me)_2$en[d]	1,2-DAP[e]	1,3-DAP[f]	SMC[g]
Glycine	1	1	0	11.21	11.79	11.01	11.12	10.13
Alanine	1	1	0	11.22	10.89	11.42	11.22	10.21
β-Phenylalanine	1	1	0	-	10.09	11.06	-	9.97
β-Alanine	1	1	0	-	-	-	-	9.75
	1	1	1					13.37
Valine	1	1	0	-	11.59	11.36	-	9.82
Proline	1	1	0	12.16	11.14	11.55	-	10.62
Iso-leucine	1	1	0		-	-	-	10.44
Methionine	1	1	0	9.14	11.27	10.37	10.31	8.75
S-Methylcysteine	1	1	0	9.38	-	10.83	10.64	8.94
Cysteine	1	1	0	-	-	-	-	14.32
	1	1	1					22.67
Serine	1	1	0	11.01	10.92	12.00	-	9.87
	1	1	-1	2.50	2.49	3.74		0.67
Threonine	1	1	0	10.96	-	11.76	10.57	9.76
	1	1	-1	2.91		3.83	2.11	0.38
Ornithine	1	1	0		13.34	13.65	-	11.23
	1	1	1		20.85	19.86		20.21
Lysine	1	1	0		11.19	11.49	-	10.87
	1	1	1		21.19	20.44		20.69
Histidine	1	1	0		14.45	14.75	-	11.50
	1	1	1					
Histamine	1	1	0		12.61	13.22	-	10.92
	1	1	1		17.03			
Aspartic acid	1	1	0		10.70	-	-	-
	1	1	1					
Glutamic acid	1	1	0		10.56	-	9.72	10.61
	1	1	1				13.60	13.99

[a]N-N = aliphatic diamine, [b]p, q and r are stoichiometric coefficients corresponding to $[Pd(diamine)(H_2O)_2]$, ligand and H^+ respectively, [c]en = ethylenediamine, data taken from reference (Lim, 1978), [d]Me_2en = N,N′-dimethylethylenediamine, data taken from reference (Mohamed and Shoukry, 2001), [e]1,2-DAP=1,2-diaminopropane, data taken from reference (Shoukry, et al., 1999); [f]1,3-DAP=1,3-diaminopropane, data taken from reference (El-Sherif et al., 2010); [g]SMC=S-Methyl-L-cysteine, data taken from reference (Shehata, et al., 2008).

Table 3. Formation constants (log β_{110}) of $[Pd(diamine)(H_2O)_2]$[a] with amino acid at 25 ^{0}C and 0.1 mol dm^{-3} $NaNO_3$.

System	p	q	r[b]	Pic[c]	AEPY[d]	AMBI[e]
Glycine	1	1	0	9.95	10.33	9.71
Alanine	1	1	0	10.89	10.46	9.98
β-Phenylalanine	1	1	0	11.05	9.86	11.01
β-Alanine	1	1	0	-	9.81	-
	1	1	1		13.37	
Valine	1	1	0	10.33	10.22	9.91
Proline	1	1	0	11.16	10.81	10.85
Iso-leucine	1	1	0	11.76	10.56	11.10
Methionine	1	1	0	9.49	9.08	9.12
S-Methylcysteine	1	1	0	10.52	9.16	10.15
Cysteine	1	1	0	-	15.11	-
	1	1	-1		19.20	
Tyrosine	1	1	0	14.61	-	-
Tryptophan	1	1	0	10.95	-	-
Serine	1	1	0	11.35	10.34	10.61
	1	1	-1	3.05	2.04	2.40
Threonine	1	1	0	10.40	10.29	-
	1	1	-1	-	2.19	
Ornithine	1	1	0	13.13	13.27	10.21
	1	1	1	20.54	20.53	18.68
Lysine	1	1	0	-	10.62	-
	1	1	1		19.49	
Histidine	1	1	0	13.36	13.37	13.14
	1	1	1		16.32	19.15
Histamine	1	1	0	13.19	12.85	10.34
	1	1	1			
Aspartic acid	1	1	0	10.02	-	-
	1	1	1			
	1	1	2			
Glutamic acid	1	1	0	-	9.19	-
	1	1	1		13.27	
	1	1	2		15.79	

[a]N-N = aromatic diamine, [b]p, q and r are stoichiometric coefficients corresponding to $[Pd(diamine)(H_2O)_2]$, ligand and H^+ respectively, [c]Pic = picolylamine, data taken from reference (El-Sherif et al., 2003), [d]AEPY = 2-aminoethylpyridine, data taken from reference (Shehata, et al., 2009), [e]AMBI=2-aminomethylbenzimidazole, data taken from reference (El-Sherif, 2006).

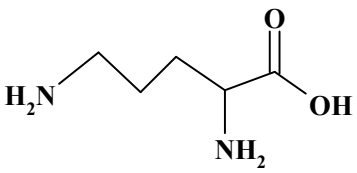
Table 4. Formation constants (log β_{110}) of $[Pd(diamine)(H_2O)_2]$[a] with amino acid at 25 ^{0}C and 0.1 mol dm^{-3} $NaNO_3$.

The pK_a value (9.20) for the Pd(SMC)-serine complex as reported in Table 3 is higher than the corresponding values of the Pd(N, N´-dimethylethylenediamine)-serine (8.43) [52] and Pd(Picolylamine)-serine complexes (8.30) (El-Sherif et al., 2003). This is due to the strong *trans* labilization effect of sulfur on the coordinated alcoholate group, and in turn hinders the induced proton ionization. This will increase the pK_a value of the alcoholate group in the case of the Pd(SMC)-serine complex.

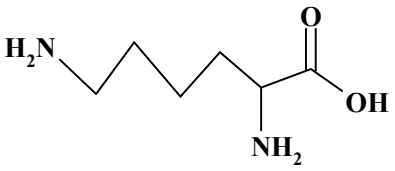
3.1.5 Interactions of $[Pd(diamine)(H_2O)_2]$ with amino acids containing amino group in the side chain

Ornithine and lysine are α- amino acids having an extra terminal- amino group. They coordinate with $[Pd(diamine)(H_2O)_2]^{2+}$ as bidentate either by the two amino groups (N,N-donor set) or glycine-like, through the α-amino and carboxylate groups (N,O-donor set). The way of coordination is depending on three factors:

1. The chelate ring size.
2. The steric effects.
3. The pH of the solution.



Ornithine



Lysine

The stability constant of the Pd(DAP)-Ornithine complex ($\log\beta_{110}$ = 13.65) is higher than those of α- amino acids. This may indicate that ornithine most likely chelates by the two amino groups at higher pH, this is being supported by the great affinity of palladium to nitrogen donor centres. Unlike ornithine, The stability constant of the Pd(DAP)-lysine complex ($\log\beta_{110}$ = 11.49) is extremely fair with those of α- amino acids. This may indicate that lysine most likely chelates by the amino and carboxylate groups (glycine-like), because chelates formed through binding with the two amino groups will form unstable eight-membered ring. The concentration distribution diagram of [Pd(AMBI)(ornithine)] complex is given in Fig. 2. It clearly shows that lysine starts to form the protonated species (111) at low pH and predominates between pH (4-9) and attains maximum concentration of ~ 98%, i.e it is the main complex species in physiological pH range. The complex species (110) predominates after pH ~ 9. The (10-1) hydrolysed species is present in very low concentration and the (10-2) species starts to form at higher pH, ca. ~ 10. Therefore, in the physiological pH range the OH^- ion doesn't compete with ornithine in the reaction with the palladium (II) complexes.

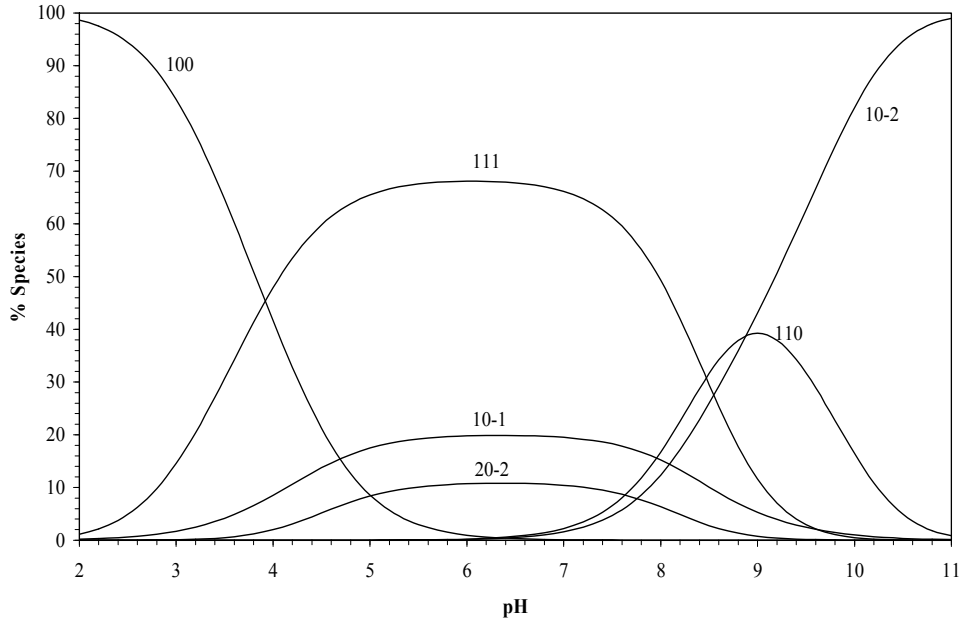
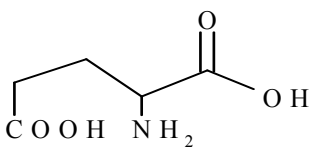


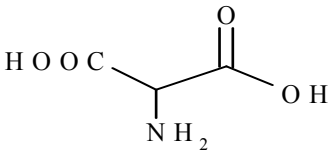
Fig. 2. Concentration distribution of various species as a function of pH in the Pd(AMBI)-Ornithine system at concemtrations of $1.25x10^{-3}$ mol-dm^{-3} for $Pd(AMBI)^{2+}$ and ornithine, I = 0.1 mol-dm^{-3} ($NaNO_3$) and T = 25 ± 0.1 ^{0}C).

3.1.6 Interactions of $[Pd(diamine)(H_2O)_2]$ with amino acids containing carboxylic acid group in the side chain

Aspartic and glutamic are α- amino acids having two carboxylic and one amino group as potential chelating sites. They coordinate with $[Pd(diamine)(H_2O)_2]^{2+}$ as bidentate either by the two carboxylate groups or by the amino and one carboxylate group. The stability constant of the aspartic and glutamic acid complexes is in the range of those for amino acids. This may reveal that both amino acids coordinate by the amino and one carboxylate group.



Glutamic acid

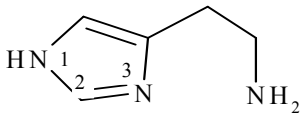


Aspartic acid

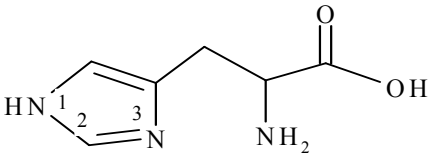
3.1.7 Interactions of [Pd(diamine)(H_2O)$_2$] with amino acids containing imidazole group in the side chain

The protonated histidine contains three dissociable protons, which can dissociate in the following sequence: carboxylic acid, imidazolium N(3)-H and side chain NH_3^+. The imidazole N(1)-H is very weekly acidic (pK_a = 14.4), and thus it does not dissociate in the measurable pH range (Burger, 1990).

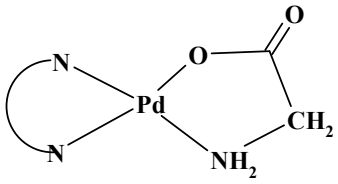
Histidine has three binding sites, provided imidazole, amino and carboxylate groups. It coordinates with [Pd(diamine)(H_2O)$_2$]$^{2+}$ as bidentate either by the α-amino group and imidazole groups (N,N-donor set) or glycine-like, through the α-amino and carboxylate groups (N,O-donor set).



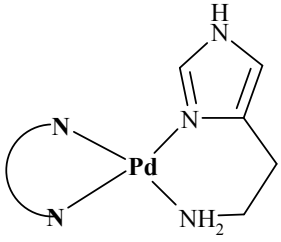
Histamine



Histidine



(Glycine-like)



(Histamine-like)

The formation constant value of the (110) species is in fair agreement with that of histamine complex, but higher than those of α-amino acids. This indicates that histidine interacts with [Pd(diamine)(H_2O)$_2$] in the same way as histamine does i.e. through the amino and imidazole.

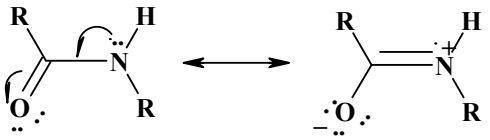
Histidine has shown to form both protonated (111) and deprotonated (110) complex species. The acid dissociation constant of the protonated species is given by the following Eq. (11).

$$pK^H = \log \beta_{111} - \log \beta_{110} \quad (11)$$

In the reaction with [Pd(AEPY)(H_2O)$_2$]$^{2+}$ species only two of the above three binding sites are involved in complex formation. The stability constants of the histidine complexes ($\log\beta_{110}$ = 13.37) is higher than that of histamine ($\log\beta_{110}$ = 12.85) by 0.52 log β units. Moreover, it is higher than those of amino acids (e.g. $\log\beta_{110}$ of glycine = 10.33) by 3.04 log units, indicating that both kinds of chelation are involved glycine-like (N,O) at lower pH and histamine-like (N,N) at higher pH. In general, histidine forms more stable complex than histamine due to the negative charge of histidine compared to neutral histamine. Furthermore, palladium may form back bonding to the π–system of imidazole ring, which brings more stable complexes.

3.2 Interactions of [Pd(diamine)(H_2O)$_2$] with peptides

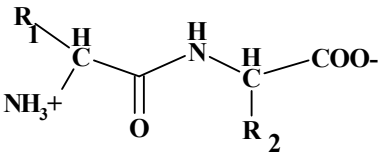
Amide bonds or groups provide the linkage between adjacent amino acids. A protein is composed of a chain of (n) amino acids contains (n-1) peptide (amide) bonds in the backbone. The tetrahedral amino nitrogen in an amino acid with pK_a ~ 9.7 loses its basicity upon reaction to give trigonal nitrogen in an amide bond. Amide groups are planar due to 40% double-bond character in the C-N bond, and the *trans* form is strongly favoured (Sigel and Martin, 1982).



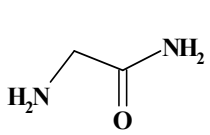
60% **40%**

The presence of the peptide linkage decreases the basicity of the amino group and the acidity of the carboxylic group.

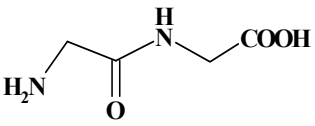
An amide group offers two potential binding atoms, the oxygen and nitrogen atoms. Throughout most of pH range, in the absence of metal ions, the amide group is neutral. It is being a very weak acid for proton loss from the trigonal nitrogen to give a negatively charged species. This very weak acidity makes quantitative equilibrium measurements very difficult. For acetamide it was reported that pK_a = 15.1, and the pK_a for glycylglycinate is 14.1.



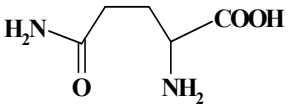
There are at least four donor groups in the dipeptide (Sigel and Martin, 1982) (amino-N, carboxylate-O, amide-N and carbonyl-O), all are capable of metal ion coordination. Because of the neutrality of the amide group, the terminal amino and carboxylate groups are the most effective binding sites for metal ions in peptides. The coordination of amide group can occur after deprotonation. Other groups may additionally be present in the side chains R_1 and R_2.



glycinamide



glycylglycine

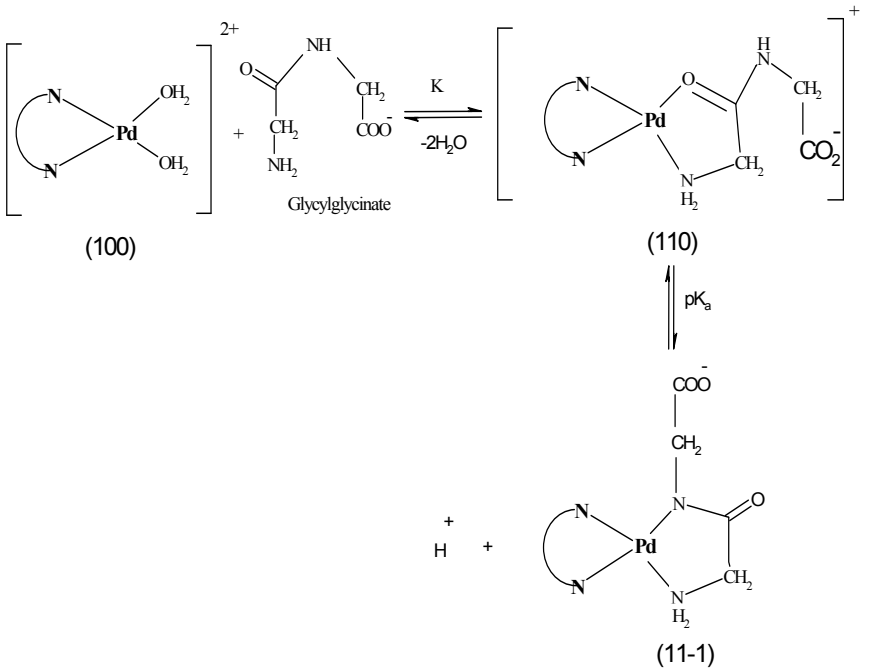


glutamine

Peptides form complexes with stochiometric coefficients 110 and 11-1 according to Scheme (4). Peptides with Pd(II) are known for promoting ionization of the peptide linkage with pK_a value calculated by Eq.10.

It is found that, most metal ions form complexes with peptides by coordinating with carbonyl oxygen of the peptide group. Only certain specific metal ions are able to promote the deprotonation of the peptide nitrogen and become coordinated with it. Among these metals are Co(II), Ni(II), Cu(II) and Pd(II). The affinity for nitrogen bonding sites over oxygen bonding sites increases from cobalt to palladium corresponding to the stability of their deprotonated complexes. This is consistent with the idea that the deprotonated amide nitrogen is a "soft" base.

It is clear that, the stability of the complex with glycylglycine (log β_{110}) is generally higher than glycinamide as indicated in Tables 5 and 6 (Shoukry et al., 1999 ; El-Sherif, 2003 ; Lim, 1978 ; Mohamed and Shoukry 2001 ; El-Sherif et al., 2010 ; Shehata et al., 2008 ; Shehata et al., 2009; El-Sherif, 2006) due to the negative charge of glycylglycinate compared to neutral glycinamide. The electrostatic interaction between dipositively charged palladium complex and the negatively charged glycylglycine would result in a general free formation energy lowering.



Scheme 4. Mode of coordination of [Pd(diamine)(H_2O)$_2$] with peptides

The pK_a value for glycinamide complex is lower than those for other peptides. This is due to the bulky substituent group on the other peptides that may hinder the structural changes when going from species 110 to 11-1 (peptide ionization). Glutamine complex has the highest stability, probably due to the presence of α-NH_2 that can coordinate first (glycine-like). The α-NH_2 of glutamine is more basic than those of other peptides resulting in more stable complexes compared to other peptides. The concentration distribution diagrams of Pd(diamine)-peptide complexes indicate that all peptides form the complex species (110) at low pH with the species (11-1) as the main product at higher pH.

System	p	q	r[b]	en[c]	$(Me)_2$en[d]	1,2-DAP[e]	1,3-DAP[f]	SMC[g]
Glycinamide	1	1	0	8.64	7.40	8.58	8.63	7.56
	1	1	-1	2.47	3.03	5.35	5.23	-3.38
Glutamine	1	1	0	10.76	10.73	11.02	9.24	8.73
	1	1	-1	9.03	5.82	2.12	-0.32	1.08
Glycylalanine	1	1	0	-	7.63	-	-	-
	1	1	-1		0.84			
Glycylglycine	1	1	0	9.60	7.75	9.41	8.01	7.73
	1	1	-1	3.76	2.69	6.02	4.24	2.61
Asparagine	1	1	0	10.46	12.31	12.79	-	8.92
	1	1	-1	6.46	4.71	6.38		2.08
Glycylleucine	1	1	0	-	8.36	7.73	-	7.69
	1	1	-1		0.01	3.30		1.98
Glycylvaline	1	1	0	-	-	-	-	7.66
	1	1	-1					2.00

[a]N-N = aliphatic diamine [b]p, q and r are stoichiometric coefficients corresponding to $[Pd(diamine)(H_2O)_2]$, ligand and H^+ respectively, [c]en = ethylenediamine, data taken from reference (Lim, 1978), [d]Me_2en = N,N′-dimethylethylenediamine, data taken from reference (Mohamed and Shoukry 2001), [e]1,2-DAP=1,2-diaminopropane, data taken from reference (Shoukry et al., 1999); [f]1,3-DAP=1,3-diaminopropane, data taken from reference (El-Sherif et al., 2010); [g]SMC=S-Methyl-L-cysteine, data taken from reference (Shehata et al., 2008).

Table 5. Formation constants (log β_{110}) of $[Pd(diamine)(H_2O)_2]$[a] with peptides at 25 ^{0}C and 0.1 mol dm^{-3} $NaNO_3$.

System	p	q	r[b]	Pic[c]	AEPY[d]	AMBI[e]
Glycinamide	1	1	0	9.30	8.01	8.63
	1	1	-1	5.73	4.16	5.23
Glutamine	1	1	0	10.02	9.11	9.24
	1	1	-1	0.36	0.47	-0.32
Glycylalanine	1	1	0	8.31	-	-
	1	1	-1	3.08		
Glycylglycine	1	1	0	8.29	8.20	8.01
	1	1	-1	4.37	3.56	4.24
Asparagine	1	1	0	10.06	9.42	8.81
	1	1	-1	2.65	1.90	-0.42
Glycylleucine	1	1	0	8.22	7.75	-
	1	1	-1	3.06	2.18	
Glycylvaline	1	1	0	7.73	-	-
	1	1	-1	2.39		
Leucylalanine	1	1	0	7.73	-	-
	1	1	-1	2.39		

[a]N-N = aromatic diamine, [b]p, q and r are stoichiometric coefficients corresponding to $[Pd(diamine)(H_2O)_2]$, ligand and H^+ respectively, [c]Pic = picolylamine, data taken from reference (El-Sherif, 2003), [d]AEPY = 2-aminoethylpyridine, data taken from reference (Shehata et al., 2009), [e]AMBI=2-aminomethylbenzimidazole, data taken from reference (El-Sherif, 2006). .

Table 6. Formation constants (log β_{110}) of $[Pd(diamine)(H_2O)_2]$[a] with peptides at 25 ^{0}C and 0.1 mol dm^{-3} $NaNO_3$.

Fitting the potentiometric data for the Pd(DAP)-glutathione system indicated the formation of complex species with the stoichiometric coefficients 110 and 111. Glutathione has various binding sites, namely oxygen atom of carboxylic group, nitrogen atom of amino group and sulphur atom of sulfhydryl group. The stability constant of the (110) complex (log β = 15.92) is higher than the ones of α-amino acids (log $\beta_{[Pd(DAP)(glycine)]}$ = 11.12). This indicates that glutathione interacts with Pd(II) ion by the amino and deprotonated SH groups and not by the amino and caboxylate group like simple α-amino acids. This is in good agreement with the fact that Pd(II) has a high affinity for S-donor ligands. The concentration distribution diagram of [Pd(DAP)(glutathione)] given in Fig. 3, shows the formation of the protonated complex 111 with a formation degree of 81% at pH 3.1. At pH 6, the complex species (110) predominates with a concentration of 99 % i.e. the reaction of $[Pd(DAP)]^{2+}$ goes to completion in the physiological pH range. This may suggest that GSH will compete with DNA for the reaction with the Pd(II) complex.

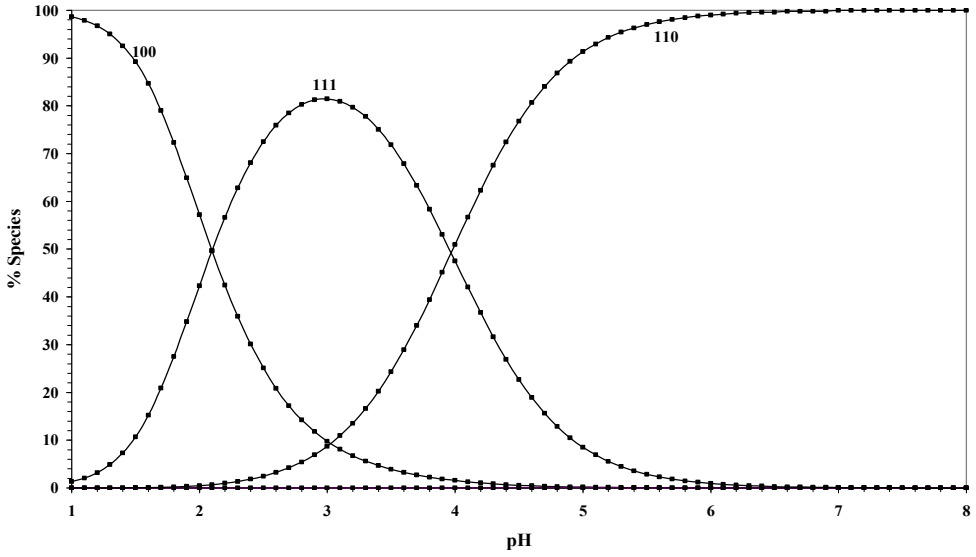
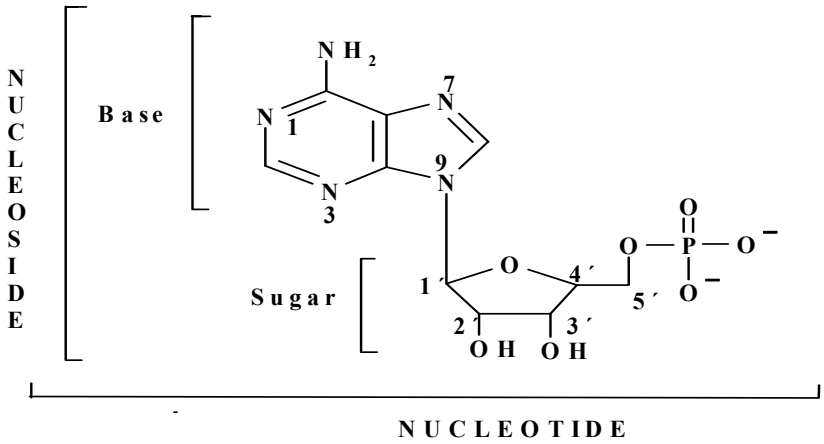


Fig. 3. Concentration distribution of various species as a function of pH in the Pd(DAP)-Glutathione system at concentrations of $1.25x10^{-3}$ mol-dm^{-3} for $Pd(DAP)^{2+}$ and glutathione, I = 0.1 mol-dm^{-3} ($NaNO_3$) and T = 25 ± 0.1 ^{0}C).

3.3 Interactions of [Pd(diamine)(H_2O)$_2$] with DNA constituents

3.3.1 Nucleosides, nucleotides and nucleic acid

Nucleosides are composed of a purine or pyrimidine base attached to the sugar ribose via the N-9 and C-1 atoms, respectively. In nucleotides, the sugar is linked to a phosphate group.



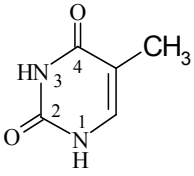
Adenosine monophosphate

The nucleic acids are polymers built up from nucleotides via phosphodiester bond formation between the 3′-OH group of one nucleotide and the 5′-OH group of the adjacent nucleotide. The sequence of the nucleotides is extremely important as it constitutes the genetic code in DNA. Different nucleotides vary in the nature of the purine and pyrimidine bases (Hay, 1985).

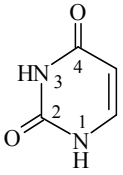
3.3.2 Ternary complexes involving DNA constituents and [Pd(diamine)(H_2O)$_2$]

The accepted models for DNA complex formation with [Pd(diamine)(H_2O)$_2$]$^{2+}$ are consistent with the formation of 1:1 and 1:2 complexes, as shown in Tables 7 and 8 (Shoukry et al.,1999; El-Sherif et al., 2003; Lim, 1978; Mohamed and Shoukry, 2001; El-Sherif, et al., 2010; Shehata, et al., 2008); Shehata, et al., 2009; El-Sherif, 2006). Generally, from Table 8, the complexes with DNA constituents are more stable (higher log β values) with bipyridine than with all other amine ligands. They are probably stabilized by intramolecular stacking between the bipyridine aromatic ring and the purine rings (Fisher and Sigel, 1980). The stability of the corresponding complexes with picolylamine has intermediate log β values, probably because in this case there is only one pyridine ring.

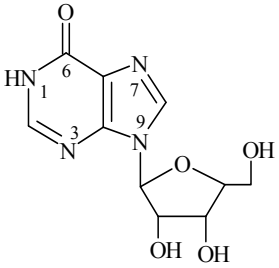
The pyrimidines, uracil and thymine have only basic nitrogen donor atoms (N3-C4O group).



Thymine



Uracil



Inosine

System	p	q	r[b]	en[c]	$(Me)_2$en[d]	1,2-DAP[e]	1,3-DAP[f]	SMC[g]
Uracil	1	1	0	8.35	8.35	8.74	8.61	8.18
	1	2	0	14.88	14.88	15.43	14.76	12.40
Uridine	1	1	0	8.70	8.70	-	-	8.10
	1	2	0	14.37	14.37			12.21
Thymine	1	1	0	-	8.56	8.90	9.02	8.63
	1	2	0		15.14	15.80	15.65	13.28
Thymidine	1	1	0	8.84	8.75	8.92	-	9.27
	1	2	0	14.69	14.53	14.84		14.10
Cytosine	1	1	0	-	-	-	-	5.73
	1	2	0					8.54
Cytidine	1	1	0	-	-	-	-	4.93
	1	2	0					8.44
Inosine	1	1	0	6.83	8.03	-	7.62	6.81
	1	1	1	-	12.40		9.69	10.16
	1	2	0	11.26	12.74			10.84
UMP	1	1	0	-	-	-	-	8.35
	1	1	1					13.62
	1	2	0					14.17
IMP	1	1	0	8.76	8.76	-	-	7.25
	1	1	1	15.26	15.26			10.60
	1	1	2	18.50	18.50			-
	1	2	0	12.31	12.31			10.51
	1	2	1		21.69			-
	1	2	2		28.45			-
GMP	1	1	0	-	-	-	-	8.20
	1	1	1					11.35
	1	2	0					14.52
Adenine	1	1	0	-	12.15	11.14	-	9.14
	1	1	1		-			11.96
	1	2	0					12.57
CMP	1	1	0	-	-	-	-	5.34
	1	1	1					7.67
	1	2	0					11.59
TMP	1	1	0		-	-	-	8.41
	1	1	1					13.53
	1	2	0					13.84

[a]N-N = aliphatic diamine bp, q and r are stoichiometric coefficients corresponding to $[Pd(diamine)(H_2O)_2]$, ligand and H^+ respectively, [c]en = ethylenediamine, data taken from reference (Lim, 1978), [d]Me_2en = N,N′-dimethylethylenediamine, data taken from reference (Mohamed and Shoukry 2001), [e]1,2-DAP=1,2-diaminopropane, data taken from reference (Shoukry et al., 1999); [f]1,3-DAP=1,3-diaminopropane, data taken from reference (El-Sherif, et al., 2010); [g]SMC=S-Methyl-L-cysteine, data taken from reference (Shehata et al., 2008).

Table 7. Formation constants (log β_{110}) of $[Pd(diamine)(H_2O)_2]$[a] with DNA constituents at 25 ^{0}C and 0.1 mol dm^{-3} $NaNO_3$.

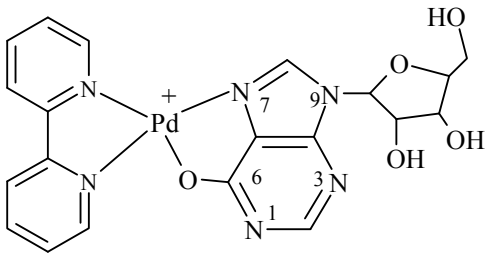
System	p	q	r[b]	Pic[c]	AEPY[d]	BPY[e]	AMBI[f]
Uracil	1	1	0	9.17	8.03	10.96	9.08
	1	2	0	15.98	14.47	17.17	12.98
	1	1	1		-	13.50	
	1	2	1		-	22.15	
Uridine	1	1	0	9.00	-	9.71	9.43
	1	2	0	14.96		16.88	13.11
	1	1	1			13.29	
	1	2	1			22.65	
Thymine	1	1	0	8.96	-	-	8.89
	1	2	0	15.62			12.94
Thymidine	1	1	0	9.17	8.25	-	9.12
	1	2	0	15.21	13.72		12.93
Cytosine	1	1	0	-	5.98	-	-
	1	2	0		8.91		
	1	1	1		10.86		
Adenosine	1	1	0	-	2.84		
	1	2	0		5.25		
Guanosine	1	1	0	-	10.48		
	1	2	0		19.03		
Adenine	1	1	0	-	9.47	11.95	-
	1	2	0		14.05	16.59	
	1	1	1		18.56	15.97	
	1	2	1		-	25.76	
	1	2	2		-	30.25	
Inosine	1	1	0	-	7.78	9.71	10.02
	1	2	0		11.64	14.89	-
	1	1	1		11.89	12.55	12.06
	1	2	1		-	20.11	-
	1	2	2		-	25.37	-
	1	1	2		-	-	14.40
IMP	1	1	0	10.42	9.18	10.17	10.05
	1	2	0	-	16.35	14.80	-
	1	1	1	16.46	13.93	16.65	15.81
	1	2	1	-	-	21.49	-
	1	2	2	-	-	28.50	-
	1	1	2	18.73	-	20.98	17.79
GMP	1	1	0	10.83	9.23	-	10.40
	1	2	0	-	13.44		-
	1	1	1	17.35	15.16		16.57
	1	2	1	-	-		-
	1	2	2	-	-		-
	1	1	2	21.01	-		19.78
CMP	1	1	0	-	5.89	11.95	-
	1	2	0		8.57	16.59	
	1	1	1		10.86	15.97	
	1	2	1		-	25.76	
	1	2	2		-	30.25	

[a]N-N = aromatic diamine, [b]p, q and r are stoichiometric coefficients corresponding to $[Pd(diamine)(H_2O)_2]$, ligand and H^+ respectively, [c]Pic = picolylamine, data taken from reference (El-Sherif, 2003), [d]AEPY = 2-aminoethylpyridine, data taken from reference (Shehata et al., 2009), [e]BPY = 2,2′-bipyridyl, data taken from reference (Shehata, 2001), [e]AMBI=2-aminomethylbenzimidazole, data taken from reference (El-Sherif, 2006)

Table 8. Formation constants (log β_{110}) of $[Pd(diamine)(H_2O)_2]$[a] with DNA constituents at 25 ^{0}C and 0.1 mol dm^{-3} $NaNO_3$.

The thymine complex is more stable than the uracil one, most probably owing to the high basicity of the N3 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 monodentates, the complexes are formed only above pH 6, supporting the view that the negatively charged nitrogen donors of pyrimidine bases are important binding sites in the neutral and slightly basic pH ranges. The purines like inosine have two metal ion binding centres N1 and N7 nitrogens. Inosine can be protonated at N7 forming a (N1H-N7H) monocation. The pK_a of N1H is 8.43 (El-Sherif, 2006) and the pK_a of N7H is 1.2 (Martin, 1985**)**. It was reported that, in the acidic pH range, N1 remained protonated, while the metal ion is coordinated to N7 i.e. these N-donors are pH dependent binding sites and there is a gradual change from N7- binding to N1-binding with increase of pH (Maskos, 1985). The results showed that inosine form the complexes 110 and 111. The speciation diagrams of Pd(diamine)-inosine complexes indicated that the species 111 is formed in acidic pH range and it corresponds to the N7 coordinated complex, while N1 nitrogen is in protonated form. 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 ligate in the deprotonated form through the N3 atom.

Several solid Pd(II) complexes of guanosine and inosine have suggested to exhibit N7-O6 chelation (Pneumatikakis, 1984 ; Pneumatikakis et al., 1988). Also, according to *ab initio* SCF calculations, N7-O6 chelation is the energetically favored bonding mode (Del Bene, 1984) ; Anwader et al., 1987).



The IR spectrum of $[Pd(BPY)(Inosine)]NO_3$ showed a shift of the ν(C=O) stretching vibration from 1700 cm^{-1} and 1676 cm^{-1} in the free inosine (which corresponds to keto-enol forms) to 1639 in the complex (Shehata, 2001). This clearly indicates the involvement of C=O in coordination. At higher pH, the N1H is deprotonated and the negative charge on N1 resonates with C=O, increasing the negative charge on the oxygen atom. This is in consistent with the large acidification of the N1H.

Inosine-5′-monophosphate (5′-IMP) forms a stronger complex with $[Pd(diamine)(H_2O)_2]$ than does inosine. The extra stabilization can be attributed to the triply negatively charged 5′-IMP^{3-} ion. The purines, inosine-5´-monophosphate and guanosine-5´-monophosphate form 110, 111 and 112 complexes. The protonated species are easily detected with aromatic amine (BPY, Pic and AMBI) rather than with aliphatic amines (en, Me_2en, 1,2-DAP and 1,3-DAP). This may be interpreted on the basis that the protonated species are stabilized through the back bonding to the π–system of aromatic rings, which makes more stable

complexes. For Pd(AMBI)-complexes, the pK_a values of the protonated species of the IMP complex (112) are 1.98 (log β_{112} - log β_{111}) and 5.76 (log β_{111} - log β_{110}). The corresponding values for GMP are 3.21 and 6.17. The former pK_a value for IMP and GMP corresponds to the N1H group while the second is assignable to the -PO_2(OH) group. The N1H groups were acidified upon complex formation by 7.23 (9.21-1.98) and 6.07 (9.28-3.21) pK units for IMP and GMP, respectively. Acidification of the N1H group upon complex formation is consistent with previous reports for IMP and GMP complexes (Sigel et al. 1994). In the fully protonated species (112), the two protons bound to N1 and the phosphate groups, exist at pH ~ 3 or lower. In the (111) complex species, which reaches its maximum concentration of 88 % at pH ~ 4.8 (Fig. 4), the single proton binds to phosphate. Therefore, monoprotonated species (111) is an N1-coordinated. The stability constant difference between the (112) and (111) complexes is 3.21, due to the pK_a of the N1 deprotonation process. The phosphate group was not acidified upon complex formation since it is too far from the coordination center. A proposed coordination process of $Pd(AMBI)^{2+}$ with 5′-GMP is reported in Scheme 5.

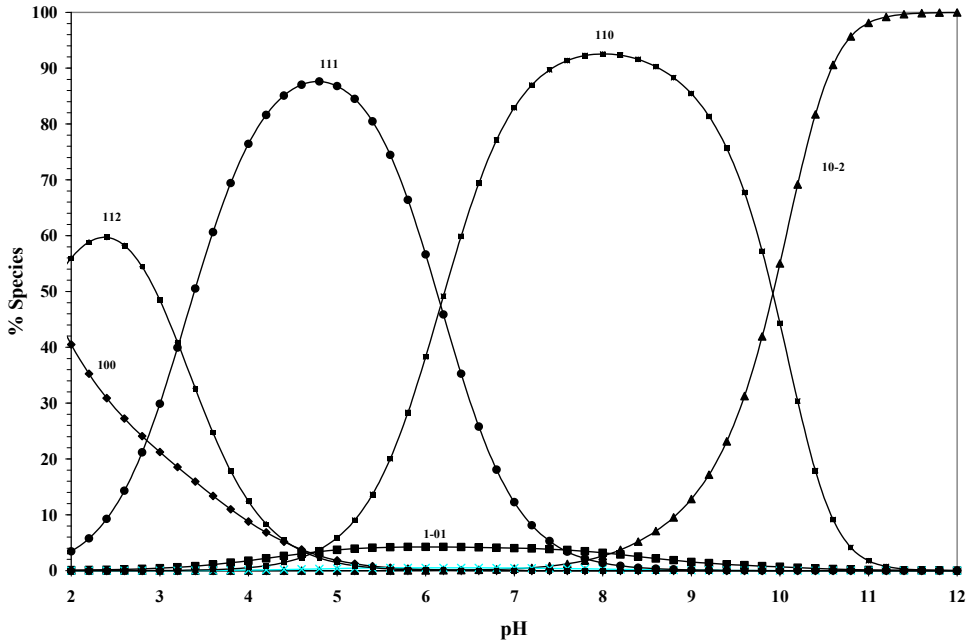
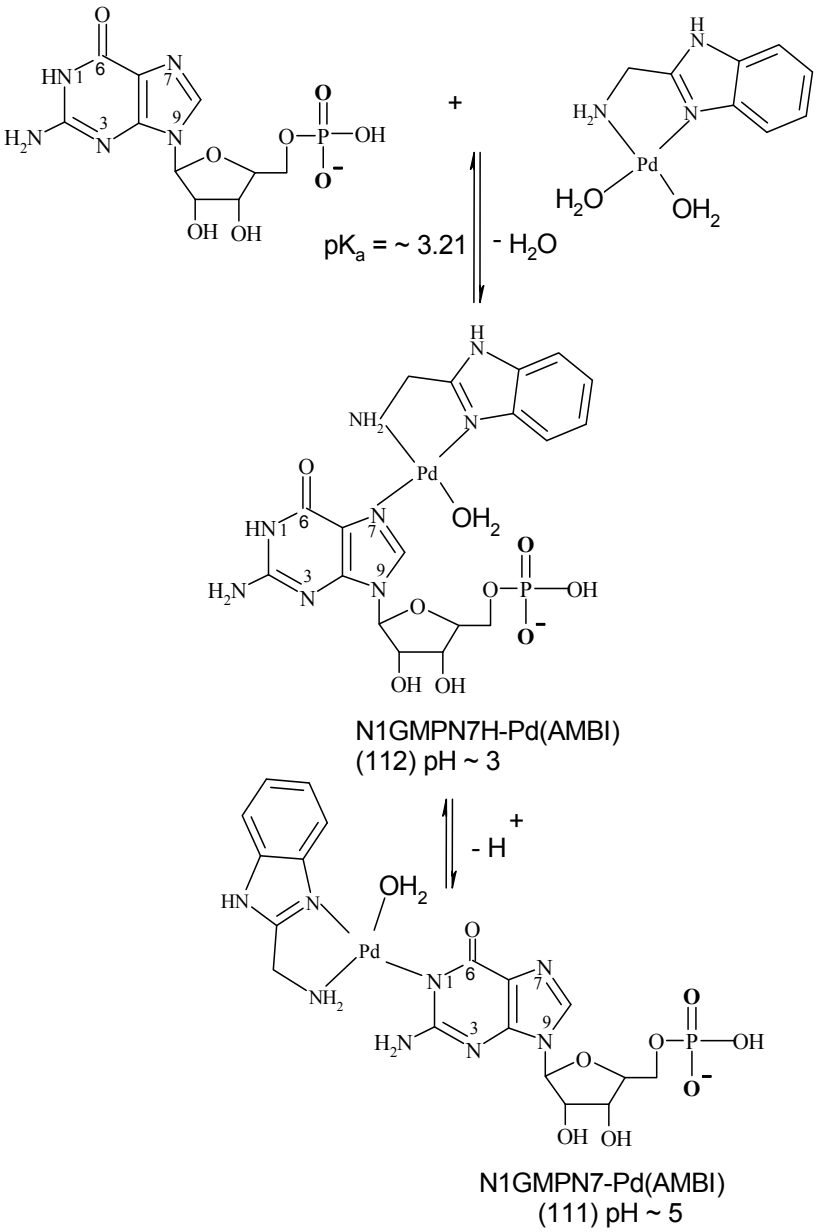


Fig. 4. Concentration distribution of various species as a function of pH in the Pd(AMBI)-5′-GMP system at concentrations of 1.25×10^{-3} mol-dm^{-3} for $Pd(AMBI)^{2+}$ and 5′-GMP, I = 0.1 mol-dm^{-3} ($NaNO_3$) and T = 25 ± 0.1 ^{0}C).



Scheme 5. Proposed coordination process of $Pd(AMBI)^{2+}$ with 5′-GMP

The IMP and GMP complexes are more stable than those of the pyrimidines. The extra stabilization can be explained on the basis of different columbic forces operating between the ions resulting from the negatively charged phosphate group. Hydrogen bonding

between the phosphate and the exocyclic amine is also thought to contribute to the higher stability of the nucleotides over that of the nucleosides (Reedik, 1992).

3.3.3 Comparison of thermodynamic and kinetic data

It is interesting to compare the the stability constants obtained from earlier kinetic results of Pd(diamine) with DNA and those estimated from potentiometric measurements. Much of the kinetic work was done in an acidic pH range in order to simplify the speciation of the system. Under these conditions, $Pd(Pic)^{2+}$ for example, binds to IMP through the N7 site, leaving the N1 site and the phosphate groups protonated. The stability constant (K) of the species formed under this condition is calculated using Eq. 12.

$$\log K = \log \beta_{112} - \log \beta_{012} \tag{12}$$

The log K value was found to be 3.52. This is comparable with the value obtained from the kinetic investigation (log K = 2.09) (Rau et al., 1997). The difference can be related to different experimental conditions (the kinetic study was performed at 10 ^{0}C with an ionic strength of 0.5 M), techniques employed and the acidity range selected for the kinetic measurements, where more than one Pd^{II} complex and/or IMP acid-base forms may contribute to the kinetic result.

It was previously shown that N-donor ligands such as DNA constituents have an affinity for $[Pd(AEPY)(H_2O)_2]^{2+}$, which may have important biological implications. However, the preference of Pd(II) to coordinate to S-donor ligands was demonstrated as shown in Tables 3 & 4. These results suggest that Pd(II)-N adducts can easily be converted into Pd-S adducts. Consequently, the equilibrium constant for such conversion is of biological significance. If we consider inosine as a typical DNA constituent (presented by HL) and cysteine as a typical thiol ligand (presented by H_2B), the equilibria involved in the complex-formation and displacement reactions are:

$$HL \rightleftharpoons H^+ + L^-$$

$$\underset{(100)}{[Pd(AEPY)]^{2+}} + L^- \rightleftharpoons \underset{(110)}{[Pd(AEPY)L]^+} \tag{13a}$$

$$\beta_{110}^{[Pd(AEPY)L]^+} = [Pd(DAP)L]^+ / [Pd(AEPY)]^{2+}[L^-] \tag{13b}$$

$$H_2B \rightleftharpoons 2H^+ + B^{2-}$$

$$\underset{(100)}{[Pd(AEPY)]^{2+}} + B^{2-} \rightleftharpoons \underset{(110)}{[Pd(AEPY)B]} \tag{14a}$$

$$\beta_{110}^{[Pd(AEPY)B]} = [Pd(AEPY)B] / [Pd(AEPY)]^{2+}[B^{2-}] \tag{14b}$$

$$[Pd(AEPY)L]^+ + B^{2-} \overset{K_{eq}}{\rightleftharpoons} [Pd(AEPY)B] + L^- \tag{15}$$

The equilibrium constant for the displacement reaction given in equation (15) is given by:

$$K_{eq} = [Pd(AEPY)B]\,[L^-] \,/\, [Pd(AEPY)L]^+[B^{2-}] \quad (16)$$

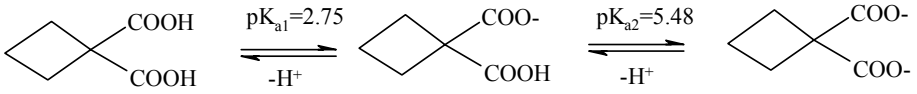
Substitution from eq. (13b) and (14b) in eq. (16) results in :

$$K_{eq} = \beta_{110}^{[Pd(AEPY)B]} / \beta_{110}^{[Pd(AEPY)L]+} \quad (17)$$

The 100 species, $[Pd(AEPY)(H_2O)_2]^{2+}$, is represented in the above equations as $[Pd(AEPY)]^{2+}$ for simplicity reasons. $\log\beta_{110}$ values for $[Pd(AEPY)(L)]^+$ and [Pd(AEPY)B] complexes taken from Tables 4 and 8 amount to 7.78 and 15.11, respectively, and by substitution in equation (17) it was found that log K_{eq} = 7.33. In the same way the equilibrium constants for the displacement of coordinated inosine by glycine and S-methylcysteine are log K_{eq} = 2.55 and 1.38, respectively. These values clearly indicate how sulfhydryl ligands such as cysteine and by analogy glutathione are effective in displacing the DNA constituent, i.e., the main target in tumour chemotherapy. Chelated cyclobutanedicarboxylate (log K_{eq} = 7.11) may undergo displacement reaction with inosine. Log K_{eq} for such a reaction was calculated as described above and amounts to 0.68. The low value of the equilibrium constant for reaction is of biological significance since it is in line with the finding that carboplatin interacts with DNA through ring opening of chelated CBDCA and not through displacement of CBDCA.

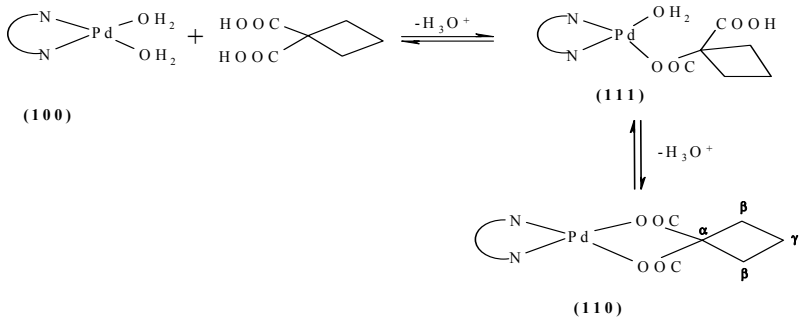
3.4 Ternary complexes involving CBDCA

Cyclobutane-1,1-dicarboxylic acid (H_2CBDCA) is a diprotic acid with pK_{a1} and pK_{a2} of 2.75 and 5.48, respectively at 25 ^{0}C and 0.1M ionic strength (El-Sherif, et al., 2010 ; Shehata, et al., 2009). The acid-base equilibria are schematized as follows:



3.4.1 Ternary complexes involving CBDCA and $[Pd(diamine)(H_2O)_2]$

The potentiometric data for H_2CBDCA complex-formation with $[Pd(diamine)(H_2O)_2]^{2+}$ were fitted considering the formation of 110 and the monoprotonated complex species 111 (Table 9) (Shoukry et al., 1999; El-Sherif et al., 2003 ; Shehata, 2001; El-Sherif et al., 2010; El-Sherif, 2006) according to Scheme (6).



Scheme 6. Coordination mode of CBDCA with $[Pd(diamine)(H_2O)_2]$

Complex	log β_{110}	log β_{111}	Reference
1,2-DAP[a]	6.05	-	(Shoukry et al., 1999)
1,3-DAP[b]	7.16	-	(El-Sherif et al., 2010)
Pic[a]	8.09	10.91	(El-Sherif et al., 2003)
AMBI[a]	7.55	10.53	(El-Sherif, 2006)
BPY[a]	8.47	11.37	(Shehata, 2001)

[a]1,2-dap,1,3-DAP, Pic, AMBI and BPY are 1,2-diaminopropane, 1,3-diaminopropane Picolylamine, 2-aminomethylbenzimidazole, and 2,2′-bipyridyl respectively at 25 ˚C and I = 0.1 M.

Table 9. Stability constants of [Pd(diamine)(CBDCA)].

The stability constants of the CBDCA complex with $[Pd(aromaticdiamine)(H_2O)_2]^{2+}$ is higher than those for $[Pd(aliphaticdiamine)(H_2O)_2]^{2+}$. Moreover, the protonated species were not observed in similar palladium complexes of CBDCA and aliphatic amines (Shoukry et al., 1999; El-Sherif et al., 2010**)** using potentiometric technique. The higher stability of CBDCA complexes with $[Pd(aromaticdiamine)(H_2O)_2]$ and the stabilization of the protonated species may be attributed to the π-acceptor properties of the pyridine rings. The pK_a of the protonated species of Pd(BPY) with CBDCA is 2.92; lower value than the one measured for $HCBDCA^-$, indicating acidification upon first chelation to Pd through one carboxylate group by 2.56 pK units (5.48-2.92). The pK_a value of this protonated species was estimated previously from UV-Vis. measurements to be ca. 2.5 at 25 °C and 0.1 M ionic strength (Shoukry et al., 1998). The involvement of the carboxylic oxygen in coordination is confirmed by the shift of the asymmetric and symmetric stretching frequencies of COO^- to lower and higher frequencies, respectively. υ_{as} and υ_s, which can be found at 1706 and 1293 cm^{-1} in H_2CBDCA are shifted to 1645 and 1354 cm^{-1} in the [Pd(BPY)(CBDCA)] complex. This corresponds to a unidentate chelation mode (Nakamoto, 1997).

3.4.2 Ring-opening of [Pd(DAP)(CBDCA)] and the formation of [Pd(DAP)(CBDCA-O)(DNA)]

The potentiometric data for the system consisting of $[Pd(diamine)(H_2O)_2]^{2+}$, CBDCA and DNA constituents were fitted assuming different models. The accepted model for the investigated DNA constituents is consistent with the formation of the 1110, 1111 and 1112 species (Table 10) (El-Sherif et al., 2003; Shehata, 2001; Mohamed and Shoukry, 2001). The results were further verified by comparing the experimental potentiometric data with the theoretically calculated curve. This supports the formation of the quaternary complex. It is interesting to notice that the quaternary complex of inosine is more stable than those of pyrimidines. This may be explained on the premise that the cyclobutane ring forms a close hydrophobic contact with the purine ring of inosine. Such contacts may contribute to the stabilization of the quaternary complexes. These studies bring to the conclusion that CBDCA is attached through one carboxylate oxygen while 5´-GMP is attached through N7 of the purine base. The same finding was obtained from an NMR investigation of the ring opening reaction of carboplatin with phosphate, chloride, and 5´-guanosine monophosphate (5´-GMP) in aqueous solution at 310 K using ^1H, ^{15}N and ^{31}P NMR spectroscopy (Frey et al., 1993). In each case a ring-opened species containing monodentate CBDCA was detected during reaction development. A structure of cis-$[Pt(NH_3)_2(CBDCA\text{-}O)(5´\text{-}GMP)]$ is proposed, taking into account the equivalence of all six cyclobutane ring protons. There is a

close hydrophobic contact between the cyclobutane ring of monodentate CBDCA and the purine ring of coordinated 5´-GMP. The reaction of carboplatin with 5´-GMP (k_{obs} 4.1 × 10^{-6} s^{-1}) was faster than that with phosphate (k_{obs} 4.3 × 10^{-7} s^{-1}) and chloride (k_{obs} 1.2 × 10^{-6} s^{-1}), or water alone (< 5 × 10^{-9} s^{-1}), suggesting that direct attack of nucleotides on carboplatin may be importance crucial step in the mechanism of action for this drug. Estimation of the concentration distribution of the various species in solution provides a useful picture of metal ion binding. To illustrate the main features observed in the species distribution plots in these systems, the speciation diagram obtained for the Pd(Pic)-CBDCA-IMP system as a representative example of [Pd(Pic)(CBDCA-O)(DNA)] is shown in Fig. 5. The Pd(Pic)-CBDCA species (1100) predominates at pH = 4.3 with maximum concentration of 73%. The Pd(Pic)-IMP species (1010) reaches the maximum concentration of 16 % at pH = 7.4. The quaternary species Pd(Pic)-CBDCA-IMP (1110) attains a maximum of 80 % in the pH range 7.6-9. This reveals that in the physiological pH range the ring opening of chelated CBDCA by DNA is quite feasible.

System	l	P	q	r[a]	$(Me)_2$en [b]	Pic[c]	BPY[d]
Uracil	1	1	1	0	16.18	14.18	18.31
	1	1	1	1	-	-	24.76
	1	1	1	2	-	-	27.05
Uridine	1	1	1	0	15.17	14.18	20.14
	1	1	1	1	-	-	26.74
	1	1	1	2	-	-	28.62
Thymine	1	1	1	0	15.71	14.34	-
	1	1	1	1	-	-	
	1	1	1	2	-	-	
Thymidine	1	1	1	0	16.26	-	-
	1	1	1	1			
	1	1	1	2			
Adenine	1	1	1	0	-	-	17.06
	1	1	1	1			23.24
	1	1	1	2			27.08
Inosine	1	1	1	0	12.29	-	16.64
	1	1	1	1	17.72		22.77
	1	1	1	2	-		25.58
GMP	1	1	1	0	-	15.07	-
	1	1	1	1		21.58	
	1	1	1	2		-	
IMP	1	1	1	0	-	14.65	16.00
	1	1	1	1		20.86	22.42
	1	1	1	2		-	27.92
	1	1	1	3		-	31.49

[a] l, p, q and r are the stoichiometric coefficients corresponding to Pd(diamine)(H2O)2], cyclobutane-1,1′-dicarboxylate, DNA and H+, respectively. [b]Me_2en = N,N′-dimethylethylenediamine, data taken from reference (Mohamed and Shoukry, 2001), [c]Pic = picolylamine, data taken from reference (El-Sherif et al., 2003), [d]BPY = 2,2′-bipyridyl, data taken from reference (Shehata, 2001).

Table 10. Formation constants (log β_{1110}) for mixed ligand complexes of [Pd(diamine)$(H_2O)_2$] with cyclobutanedicarboxylic acid and some DNA units at 25 ^{0}C and 0.1M ionic strength.

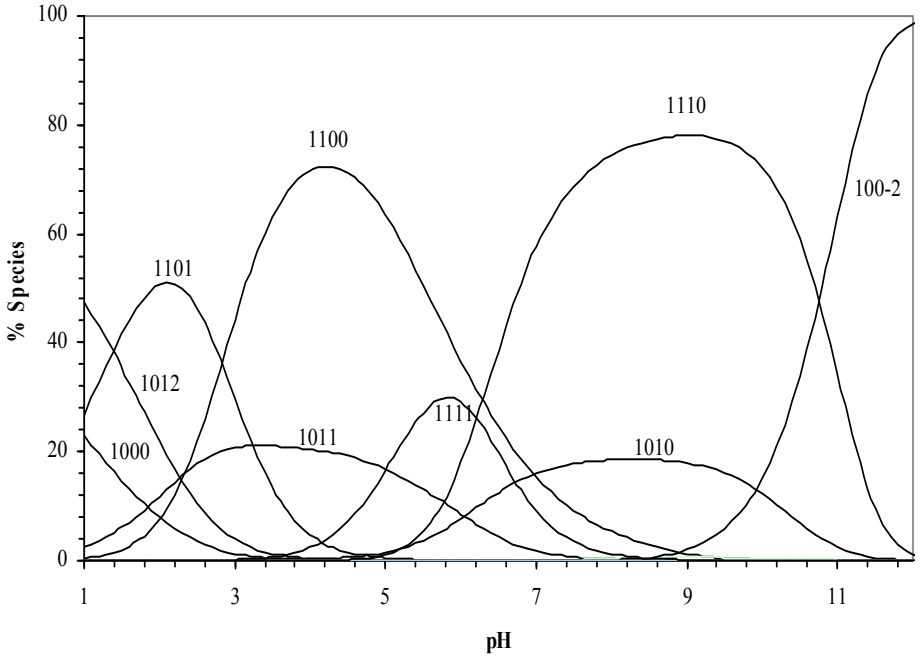


Fig. 5. Concentration distribution of various species as a function of pH in the Pd(Pic)-CBDCA-IMP system at concentrations of 1.25×10^{-3} mol-dm^{-3} for $Pd(Pic)^{2+}$; CBDCA and IMP, I = 0.1 mol-dm^{-3} ($NaNO_3$) and T = 25 ± 0.1 ^{0}C).

4. Effect of solvent on the stability constants

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 (Rees, 1980 ; Rogersa et al., 1985; Akerlof and Short, 1953). 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(Pic)-CBDCA and Pd(1,3-DAP)-CBDCA complex formation, taken as a typical example for [Pd(aliphaticdiamine)CBDCA] and [Pd(aromaticdiamine)CBDCA] respectively, in dioxane-water solutions of different compositions could be of biological significance. In order to characterize the formation equilibria of the Pd(diamine)-CBDCA complex in dioxane-water solutions, all other equilibria involved, namely acid-base equilibria of CBDCA and $[Pd(diamine)(H_2O)_2]^{2+}$, have to be studied in the same solvent. The equilibrium constants are reported in Table 11 (El-Sherif et al., 2003, 2010). The hydrolysis of $Pd(diamine)^{2+}$ 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 $[Pd(diamine)(H_2O)_2]^{2+}$ increase linearly with increasing of dioxane concentration. This may be correlated with the ability of a relatively low dielectric solvent to increase the electrostatic attraction between the proton and the ligand anion in

case of CBDCA and between a proton and the hydrolysed form of Pd(II) species. The variation in the stability constant of the $[Pd(diamine)(H_2O)_2]^{2+}$ complex with CBDCA as a function of solvent composition, is shown in Fig. 6. The stability constant for the Pd(diamine)-CBDCA complex increases linearly with increasing dioxane concentration. This is explained in terms of complex formation involving oppositely charged ions as in the Pd(diamine)-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 $Pd(diamine)^{2+}$ will be more favoured in biological environments of lower dielectric constant.

% Dioxane	p	q	r[a]	Pic[b]	1,3-DAP[c]
12.5	1	1	0	8.93	8.13
25	1	1	0	9.36	8.57
37.5	1	1	0	9.89	8.98
50	1	1	0	10.58	9.48
62.5	1	1	0	10.99	10.13

[a]p, q and r are stoichiometric coefficients corresponding to $[Pd(diamine)(H_2O)_2]$, ligand and H^+ respectively, [b]Pic = picolylamine, data taken from reference (El-Sherif et al., 2003), [c]1,3-DAP = 1,3-diaminopropane, data taken from reference (El-Sherif et al., 2010).

Table 11. Effect of dioxane on the formation constant (log β_{110}) of $[Pd(diamine)(H_2O)_2]$ with CBDCA at 25 ^{0}C and 0.1 mol dm^{-3} $NaNO_3$.

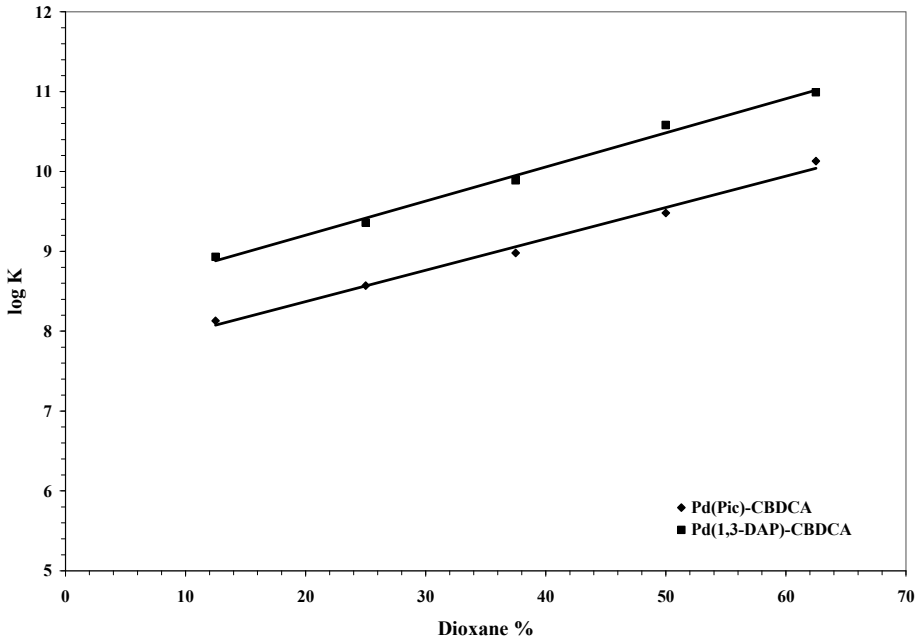
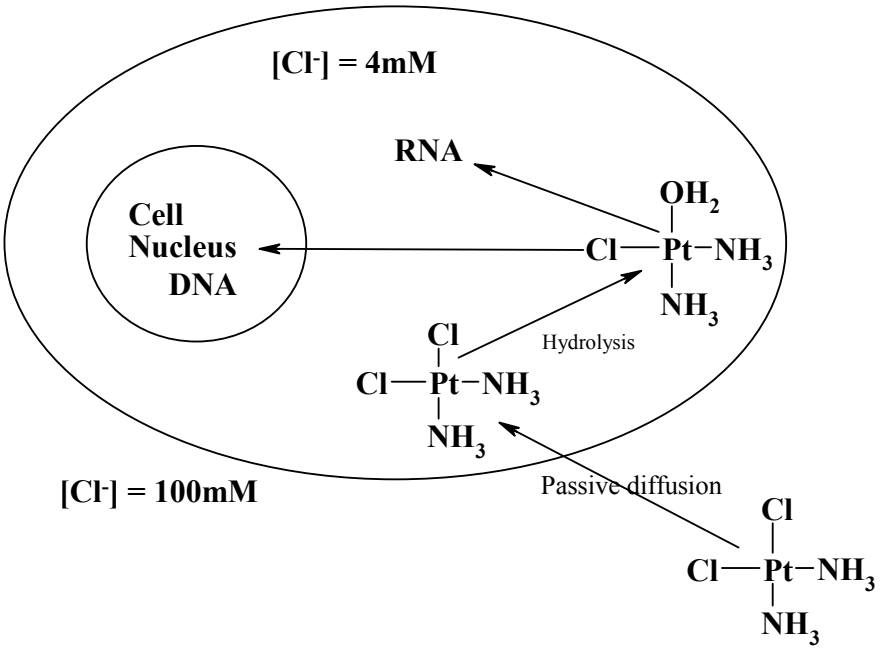


Fig. 6. Effect of dioxane concentration on the stability of the Pd(diamine)-CBDCA system and logK refers to the 110 species.

5. Effect of chloride on the stability constants

Carboplatin or *Cis*-diamine(1,1-cyclobutanedicarboxylate)platinum(II), is a clinically successful second generation platinum complex. When Pt antitumor drugs are injected into the blood, it is crucial that they reach their final target, the intracellular DNA, without reacting with other nucleophiles within the cytoplasm. In human blood plasma the chloride content is quite high (~ 100 mM), so that hydrolysis of *cis*-$[PtCl_2(NH_3)_2]$ is less likely to occur than within the cell where the chloride concentration is as low as 4 mM (Rosenberg, 1980). The high chloride concentration allows the neutral complex, *cis*-$[PtCl_2(NH_3)_2]$, to flow almost unchanged through the blood. It then diffuses through the cell membrane and finally hydrolyzed to give more reactive aquated species as shown in scheme 7. Due to the lower chloride concentration the aquated complexes can react with DNA.



Scheme 7. The cellular uptake of cisplatin and its targets.

A realistic extrapolation of the present study to biologically relevant conditions will require information on the effect of the chloride concentration on the reported stability constants. The reactivity of CBDCA toward the different Pd(II) species increases markedly when chloride ions of $Pd(AMBI)Cl_2$ are replaced successively by one and two water molecules. A similar qualitative conclusion has been reached in the case of $Pt(en)Cl_2$ by Lim and Martin (Lim and Martin, 1976), based on equilibrium distribution of Pt(en) and on rates of reactions of pyridine with Pt(dien) complexes.

The equilibrium constants for Pd(AMBI)-CBDCA complex obtained for different chloride ion concentrations, keeping the ionic strength constant at 0.30 mol-dm^{-3}, are summarized in

Table 12 (El-Sherif et al., 2003; El-Sherif, 2006). The stability constants of the Pd(AMBI)-CBDCA complex, tend to decrease while increasing the chloride ion concentration. This can be accounted for on the basis that the concentrations of the active species, the mono- and the diaqua complexes, decrease with increasing [Cl-]; this will in turn affect the stability of the complexes formed. The variation in stability constant of the $Pd(diamine)^{2+}$ complex with CBDCA as a function of Cl^- ion concentration, is shown in Fig. 7.

[Cl-]/M	p	q	r[a]	Pic[b]	AMBI[c]
0.05	1	1	0	3.95	3.55
0.10	1	1	0	3.56	3.15
0.15	1	1	0	3.30	2.80
0.20	1	1	0	3.00	2.40
0.25	1	1	0	2.62	-

[a]p, q and r are stoichiometric coefficients corresponding to $[Pd(diamine)(H_2O)_2]$, ligand and H^+ respectively, [b]Pic = picolylamine, data taken from reference (El-Sherif et al., 2003), [c]AMBI = 2-aminomethylbenzimidazole, data taken from reference (El-Sherif, 2006).

Table 12. Effect of chloride on the formation constant (log β_{110}) of $[Pd(diamine)(H_2O)_2]$ with CBDCA at 25 ^{0}C and 0.3 mol dm^{-3} (KCl + $NaNO_3$).

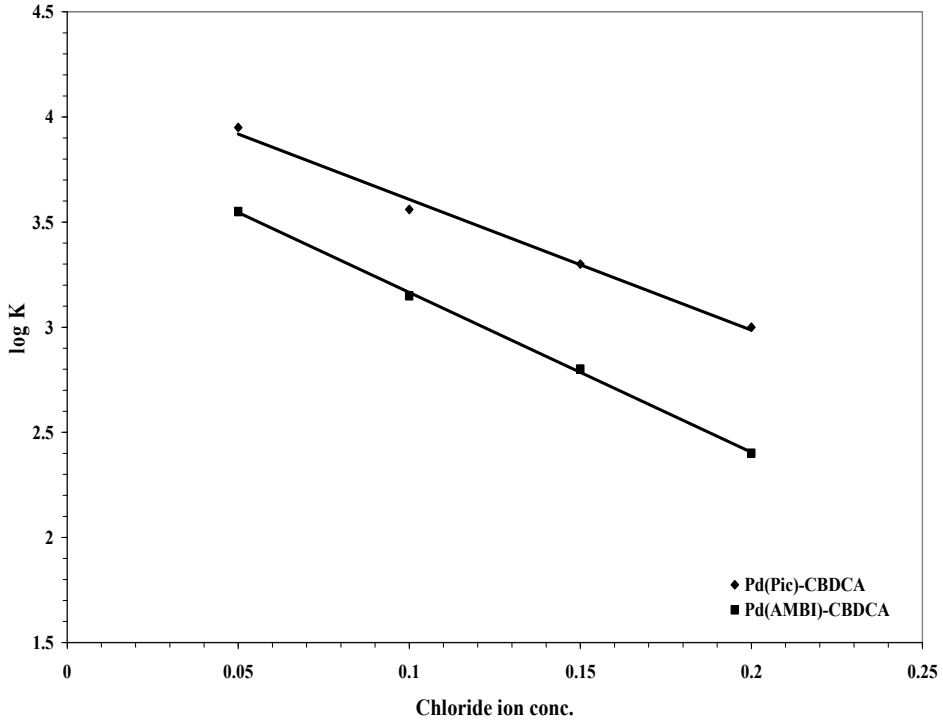


Fig. 7. Effect of chloride ion concentration on the stability of the Pd(diamine)-CBDCA system and logK refers to the 110 species.

6. Biological activity of mixed ligand Pd(II) complexes with biologically active ligands

Four new palladium(II) and platinum(II) complexes of formula $[M(BPY)(AA)]^+$ (where BPY is 2,2′-bipyridylamine, AA is an anion of glycine or L-alanine, and M is Pd(II) or Pt(II) have been synthesized (Paul et al., 1993) and characterized with amino acids bound as bidentate ligands. These complexes are 1:1 electrolyte in conductivity water. Among the studied complexes, the two L-alanine complexes show ID_{50} values against lymphocytic leukemia cells lower than cis-diammine dichloroplatinum(II), whereas the two glycine complexes show ID_{50} values higher than cisplatin. The interaction of calf thymus DNA with the above complexes shows significant spectral changes in the presence of [Pt(BPY)(gly)]Cl, [Pd(BPY)(ala)]Cl, and [Pt(BPY)(ala)]Cl and the binding mode between these complexes and DNA seems to be noncovalent.

Nine palladium(II) complexes of the formula $[Pd(BPY)(AA)]^{n+}$ (where BPY is 2,2′-bipyridylamine, AA is an anion of L-cysteine, L-aspartic acid, L-glutamic acid, L-methionine, L-histidine, L-arginine, L-phenylalanine, L-tyrosine, L-tryptophan, n = 0 or1) have been synthesized by the interaction of $[Pd(BPY)Cl_2]$ with an appropriate sodium salt of amino acids in water. The Pd(II)-complexes have shown growth inhibition against L1210 lymphoid leukemic, P388 lymphocytic leukemic sarcoma 180 and Ehrlich ascites tumor cells. Some of these complexes show ID_{50} values comparable to *cis*-platin (Puthraya et al.,1986).

The amino acids (AA) complexes of $[Pt(phen)(AA)]NO_3 \cdot xH_2O$ and $[Pd(phen)(AA)]NO_3 \cdot x H_2O$ have been prepared by the interaction of palladium and platinum complexes, $[Pt(phen)Cl_2]$ and $[Pd(phen)Cl_2]$ with salts of amino acids in methanol and characterized by ^1H NMR and IR spectroscopy which confirmed the formation of a very large variety of compounds (Jin et al., 2000). All of these new compounds have been isolated and tested for cytotoxicity on Molt-4, a human leukaemia cell line. The IC_{50} values of $[Pt(phen)(Pro)]Cl \cdot 2H_2O$ and $[Pd(phen)(Asp)]Cl \cdot 1.5H_2O$ are 9.8 and 7.31 μM, respectively, exhibiting a significant activity which is close to *cis*-$[PtCl_2(NH_3)_2]$.

Palladium(II) complexes of type $[Pd(phen)(AA)]^+$ (where AA is an anion of glycine, L-alanine, L-leucine, L-phenylalanine, L-tyrosine, L-tryptophan, L-valine, L-proline, or L-serine), have been synthesized. These palladium(II) complexes have been characterized by ultraviolet-visible, infrared, and ^1H NMR spectroscopy. They have also been screened for cytotoxicity in P388 lymphocytic cells. Only two complexes, $[Pd(Phen)(Gly)]^+$ and $[Pd(phen)(Val)]^+$, showed a comparable cytotoxictty with cisplatin (Mital, et al., 1991).

Five new palladium(II) complexes of the formula $[Pd(AMBI)(AA)]^{n+}$ (where AMBI = 2-aminomethyl benzimidazole, AA is an anion of glycine, alanine, cysteine, methionine and threonine) have been synthesized (El-Sherif, 2011). These palladium(II) complexes have been ascertained by elemental, molar conductance, infrared and ^{1}HNMR spectroscopy. The isolated Pd(II)-complexes were screened for their antibacterial and cytotoxic activities and the results are reported and discussed. The activity of these compounds against Staphylococcus pyogenes decreased in the order Tavanic (standard) > [Pd(AMBI)(Met)]~ [Pd(AMBI)(Cys)] > [Pd(AMBI)(Ser)] > [Pd(AMBI)(Ala)] > [Pd(AMBI)(Gly)] and against E. coli decreased in the order Tavanic ~ [Pd(AMBI)(Met)] > [Pd(AMBI)(Cys)] > [Pd(AMBI)(Ser)] > [Pd(AMBI)(Ala)] > [Pd(AMBI)(Gly)] under experimental conditions. Cytotoxic study of the compounds against colon carcinoma (HCT116) and larynx carcinoma

(HEP2) cells indicate that, [Pd(AMBI)(Met)]Cl.H_2O complex shows significant activity against (HCT116) cells with IC_{50} value of 0.74 μg/ml, while [Pd(AMBI)(Cys)] complex shows significant activity against (HEP2) with IC_{50} value of 0.60 μg/ml. These results confirm the chemotherapeutically significance of these compounds (El-Sherif, 2011).

7. Conclusions

In this review, a detailed survey of the formation equilibria of $[Pd(diamine)(H_2O)_2]^{2+}$ with ligands of biological significance is presented. The main conclusions may be summarized as follows:

1. The stability constants of the hydroxo complexes are higher for $[Pd(aromaticdiamine)(H_2O)_2]^{2+}$ than $[Pd(aliphaticdiamine)(H_2O)_2]^{2+}$, suggesting that the presence of aromatic residues increases the affinity of palladium(II) for hydrolysis.
2. Combining of stability constants data of such diaqua-complexes with amino acids, peptides and DNA constitutents, it would be possible to calculate the equilibrium distibution of the metal species in biological fluids where all types of ligands are present simultaneously. This would constitute a powerful starting point for understanding the mode of action of such metal species under physiological conditions.
3. Amino acids form highly stable complexes, with the substituent on the α- carbon atom possessing a significant effect on the stability of the formed complex. The thioether group in S-methylcysteine increase the stability constant of its complex due to the highest coordination potentiality of the sulphur atom. The imidazole group in histidine increases the stability of the complex due to high affinity of PdII to the nitrogen donor group. On the other hand the extra carboxylic group in aspartic acid does not contribute to the stability of the formed complex, as the extra-carboxylic group is not competing with the amino group in complex formation.
4. The present study clearly shows clearly that $[Pd(diamine)(H^2O)_2]^{2+}$ complex can form strong bonds with peptides and promote easy 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 condition (pH 6-7), the peptides would coordinate to $[Pd(diamine)(H^2O)_2]^{2+}$ in entirely different ways. Glutaminate exists solely in its protonated form, whereas the other peptides are present entirely in the deprotonated form. Furthermore, the slight difference in the side chain of the peptides seem to produce dramatic differences in their behaviour toward the Pd(II) complex.
5. Anti-tumour Pt(II) amines are usually administrated as cisdichloro complexes. This form persists in human blood plasma because of its high chloride content (0.1 M). The net zero charge on the complex facilitates its passage through cell walls. Within many cells the chloride ion concentration is much lower (only ca. 4 mM), giving more reactive aquated species. Due to the lower chloride concentration, the complex diffuses through the cell membrane and is then hydrolyzed to give the more reactive aquated complexes which can then react with DNA
6. The reactivity of CBDCA toward the different Pd(II) species increases markedly when chloride ions of $Pd(diamine)Cl_2$ are replaced successively by one and two water molecules. A similar qualitative conclusion has been reached in the case of $Pt(en)Cl_2$ by Lim and Martin, based on equilibrium distribution of en Pt(II) and on rates of reactions of pyridine with dien Pt(II) complexes (Lim, and Martin, 1976).

7. Pd(diamine)-CBDCA complex formation is more favoured in biological environments of lower dielectric constant.
8. Hopefully that these data will bring a significant contribution in carrying out mechanistic studies in biological media.

8. Aknowledgement

The author thanks the Chemistry Department, Cairo University, Faculty of Science, Egypt for financial support and Prof. Dr. M.M. Shoukry for his efforts and co-operation. The author is also grateful to the chairman of Chem. Department (Prof. Dr. M. Badway) and the Dean of Faculty of Science (Prof. Dr. Gamal Abd El-Nasar for their constant encouragement for this work.

9. Abbreviations

Pic = Picolylamine (2-Aminomethylpyridine); BPY = 2,2′-bipyridyl; 1,3-DAP = 1,3-diaminopropane; 1,2-DAP = 1,2-diaminopropane**;** AEPY = 2-aminoethyl pyridine; UMP = Uridine-5′-monophosphate; TMP = Thymidine-5′-monophosphate; GMP = Guanosine-5′-monophosphate; CMP = Cytidine-5′-monophosphate; IMP = Inosine-5′-monophosphate; Phe = Phenylalanine; Ala = Alanine.

10. References

Akerlof, G. Short, O.A. (1953) The dielectric constant of dioxane-water mixtures between 0 and 80 degrees-correction. J Am Chem Soc. 75, 6357.

Anwader, E.H.S., Probst, M.M. and Rode. B.M. (1987) Investigations of Zn(II) complexes with DNA/RNA bases by means of quantum chemical calculations. Inorg Chim Acta. 137, 203-208.

Appleton, T.G. (1997) Donor atoms preferences in complexes of Pt(II) and Pd(II) with amino acids and related molecules. Coord Chem Rev 166, 313-359.

Appleton, T.G., Hall, J.R. and Ralph, S.F. (1986) ^{15}N and ^{195}Pt NMR-study of the effect of chain-length, n, on the reactions of amino-acids, $^{+}NH_3(CH_2)NCO_2$ (N = 1, 2, 3), with platinum(II) ammine complexes. Aus J Chem. 39(9), 1347-1362.

Beltrán, J.L., Codony, R. and Prat, M.D. (1993) Evaluation of stability constants from multi-wavelength absorbance data: program STAR. Anal Chim Acta. 276, 441.

Bernhard, S.A. (1986). The structure and function of enzymes, Benjamin WA, New York, ch. 8.

Burger, K., in Burger, K. (Ed.) (1990) Bio-coordination chemistry: coordination equilibria in biological active systems, Ellis Horwood Ltd., England.

Bóka, B., Nagy, Z. and Sóvágo, I. (2001) Solution equilibria and structural characterisation of the palladium(II) and mixed metal complexes of peptides containing methionyl residues. Journal of Inorganic Biochemistry 83, 77-89.

Britten, J.F., Lock, C.J.L., Pratt, W.M.C. (1982) The structures of chloro(diethylenetriamine) platinum(II) chloride and (diethylenetriamine)nitratoplatinum(II) nitrate and some comments on the existence of Pt^{II}-OH_2 and Pt^{II}–OH bonds in the solid state. Acta Crystallogr Sect B 38(8), 2148-2155.

Byabartta, P., Santra, P.K., Misra, T.K., Sinha, C. and Kennard, C.H.L., (2001) Synthesis, spectral characterisation, redox studies of isomeric dichloro-bis-[1-alkyl-2-(naphthyl-(α/β)-azo)imidazole]ruthenium(II). Single crystal X-ray structure of blue-green dichloro-bis-[1-ethyl-2-(naphthyl-α-azo)imidazole] ruthenium(II). Polyhedron. 20, 905.

Chandler, J.P., Thomson. R.E., Spivey, H.O. and Li, EL-F (1984) An improved computer program for calculating formation constants of ligand complexes from pH data. Anal Chim Acta. 162, 399-402.

Das, D., Nayak, M.K. and Sinha, C. (1997). Chemistry of azoimidazoles. Synthesis, spectral characterization and redox studies of N(1)-benzyl-2-(arylazo) imidazolepalladium(II) chloride. Transition Met Chem. 22,172-175.

Das, D. and Sinha, C. (1998). Synthesis, spectral and electrochemical characterization of (dithiocarbamato) (arylazoimidazole)Palladium(II) perchlorates. Transition Met Chem. 23, 309-311

Del Bene, J.E. (1984) Molecular orbital study of the lithium (1+) of the DNA bases J Phys Chem. 88, 5927-5931.

El-Sherif, A.A., (2006) Mixed-Ligand Complexes of 2-(aminomethyl)benzimidazole palladium(II) with various biologically relevant ligands. J Solution Chem. 35, 1287-1301.

El-Sherif, A.A., Shoukry, M.M., El-Bahnasawy, R.M. and Ahmed, D.M. (2010) Complex formation reactions of palladium(II)-1,3-diaminopropane with various biologically relevant ligands. Kinetics of hydrolysis of glycine methyl ester through complex formation. Cent Eur J Chem. 8(4), 919-927.

El-Sherif, A.A. (2011) Synthesis and characterization of some potential antitumor palladium(II) complexes of 2-aminomethylbenzimidazole and amino acids. J Coord. Chem, 64(12) 2035-2055.

El-Sherif, A.A., Shoukry, M.M. and van Eldik, R. (2003) Complex-formation reactions and stability constants for mixed-ligand complexes of diaqua(2-picolylamine)palladium(II) with some bio-relevant ligands. J. Chem Soc Dalton Trans, 1425-1432.

Faggiani, R., Lippert, B., Lock, C.J.L., and Rosenberg, B. (1977) Hydroxo-bridged platinum(II) complexes. 1. Di-.mu.-hydroxo-bis[diammineplatinum(II)] nitrate, $[(NH_3)_2Pt(OH)_2Pt(NH_3)_2](NO_3)_2$. Crystalline structure and vibrational spectra. J Am Chem Soc. 99, 777.

Faggiani, R., Lippert, B., Lock, C.J.L., and Rosenberg, B., (1977) Hydroxo-bridged platinum(II) complexes. 2. Crystallographic characterization and vibrational spectra of cyclo-tri-.mu.-hydroxo-tris[cis-diammineplatinum(II)]nitrate. Inorg Chem. 16, 1192-1196.

Faggiani, R., Lippert, B., Lock, C.J.L. and Rosenberg, B. (1978) Hydroxo-bridged platinum(II) complexes. 3. Bis[cyclo-tri-.mu.-hydroxo-tris(cis-diammineplatinium(II)] trisulfate hexahydrate. Crystallographic characterization and vibrational spectra. Inorg Chem. 17, 1941-1945.

Fisher, B.E. and Sigel, H. (1980) Ternary complexes in solution. Intramolecular hydrophobic ligand-ligand interactions in mixed ligand complexes containing an aliphatic amino acid. J Am Chem Soc. 102, 2998-3008.

Frassineti, C., Ghelli, S., Gans, P., Sabatini, A., Moruzzi, M.S. and Vacca, A. (1995) Nuclear magnetic resonance as a tool for determining protonation constants of natural polyprotic bases in solution. Anal Biochem. 231, 374-382.

Freeman, H.C. and Golomb, M.L. (1964) The crystal structure of trans-bisglycinatoplatinum(II) $Pt(NH_2CH_2COO)_2$. Acta Cryst Sect B 25(6),1203-1206.

Frey, U., Ranford, J.D. and Sadler P.J. (1993) Ring-opening reactions of the anticancer drug carboplatin: NMR characterization of cis-[$Pt(NH_3)_2$(CBDCA-O)(5'-GMP-N7)] in solution. Inorg Chem. 32, 1333-1340.

Galanski, M., Jakupec, M.A. and Keppler, B.K. (2005) Update of the preclinical situation of anticancer platinum complexes: Novel design strategies and innovative analytical approaches. Curr Med Chem. 12, 2075-2094.

Gampp, H., Maeder, M., Meyer, C.J and Zuberbühler, A. (1985) Calculation of equilibrium constants from spectroscopic data-II:Specfit: two user-friendly in basic and standard fortran. Talanta 32, 251-264.

Gans, P. Sabatini, A. and Vacca, A. (1976), An improved computer program for the computation of formation constants from potentiometric data. Inorg Chim Acta. 18, 237-239.

Gans, P., Sabatini, A. and Vacca, A. (1985) SUPERQUAD, an improved general program for computation of formation constants from potentiometric data. J Chem Soc, Dalton Trans. 1195.

Gill, D.S. (1984) in Platinum Coordination Complexes in Cancer Chemotherapy, Hacker, M.P., Douple, E.B., Krakoff, I.H., Eds. Nijhoff, Boston, pp. 267-278.

Guo, Z. Sadler, P.J. (2000) Medicinal inorganic chemistry. Adv. Inorg. Chem 49: 183-306.

Gordon, W.E. (1982) Data analysis for acid-base titration of an unknown solution. Anal Chem. 54 (9), 1595-1601.

Hay, R.W. (1985) "Bio-inorganic chemistry", Ellis Horwood Ltd., John Wiley and Sons NY.

Heim, M.E. (1993) in: B.K. Keppler, (Ed.), Metal complexes in cancer chemotherapy, VCH, Weinheim, p. 9.

Hohmann, H., Hellquist, B. and van Eldik, R. (1991) Effect of steric hindrance on kinetic and equilibrium data for substitution reactions of diaqua(N-substituted Eehylenediamine)palladium(II) with chloride in aqueous solution. Inorg Chim Acta. 188, 25-32.

Jin, V.X. and Ranford, J.D. (2000) Complexes of platinum(II) or palladium(II) with 1,10-phenanthroline and amino acids. Inorg Chim Acta. 304, 38.

Legget, D.J. (Ed.) (1985) Computational methods for the determination of formation constants, Plenum Press, New York.

Lim, M.C. (1978) Mixed-ligand complexes of Pd(II). Part(3). Diaqua(ethylenediamine)Pd(II)-complexes of L-amino acids. JCS Dalton. 726-728.

Lim, M.C., Martin, R.B. (1976) The Nature of cis-ammine Pd(II) and antitumor in aqueous solutions. J Inorg Nucl Chem 38, 1911-1914

Maskos, K. (1985) Spectroscopic studies on Cu(II)-Inosine system, J Inorg Biochem. 25,1-14.

Martin, R.B. (1983) in Platinum, Gold and other chemotherapeutic agents: Chemistry and Biochemistry, ed. Lippard, S.J., ACS Symposium Series, Wiley, New York, 209, 231.

Martin, R.B. (1985) Nucleoside sites for transition metal-ion binding, Accounts Chem Research 18(2), 32-38.

Martin, R.B. (1999) Platinum complexes: Hydrolysis and binding to N(7) and N(1) of purines, in Cisplatin. ed. Lippert, B., Wiley-VCH, Weinheim. 183-205.

Misra, T.K., Das, D., Sinha, C., Ghosh, P.K. and Pal. C.K. (1998) Chemistry of azoimidazoles: Synthesis, spectral characterization, electrochemical studies, and X-ray crystal structures of isomeric dichloro bis[1-alkyl-2-(arylazo)imidazole] complexes of ruthenium(II). Inorg Chem. 37,1672-1678.

Mital, R., Srivastava, T.S., Parekh, H.K., Chitnis, M.P. (1991) Synthesis, characterization, DNA binding, and cytotoxic studies of some mixed-ligand palladium(II) and platinum(II) complexes of α-diimine and amino acids. J Inorg Biochem. 41, 93-103.

Mohamed, M.M.A. and Shoukry, M.M. (2001) Complex formation reactions of (N,N'-dimethylethylenediamine) palladium(II) with various biologically relevant ligands. Polyhedron. 20, 343-352.

Momekov, G. Bakalova, A. and Karaivanova, M. (2005) Novel approaches towards development of non-classical platinum-based antineoplastic agents: Design of platinum complexes characterized by an alternative DNA-binding pattern and/or tumor-targeted cytotoxicity. Curr Med Chem. 12(19), 2177-2191.

Motekaitis, R.J. and Martell, A.E. (1988). The determination and use of stability constants, VCH, Ne.w York.

Meloum, M., Havel, and J., Hgfeldt, E. (1994) Computation of solution equilibria, Ellis Horwood, Chichester.

Nagy, Z. and Sóvágó, I. (2001) Thermodynamic and structural characterization of the complexes formed in the reaction of $[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(pic)(H_2O)_2]^{2+}$ with N-alkyl nucleobases and N-acetylamino acids. J Chem Soc, Dalton Trans. 2467.

Nakamoto, K. (1997) Infrared and raman spectra of inorganic and coordination compounds, 5th edit., part B applications in coordination, organometallic, and bioinorganic chemistry, Wiley Interscience, New York.

Nelson, D.J., Yeagle, P.L., Miller, T.L. and Martin, R.B., (1976) Carbon-13 magnetic resonance spectra of nucleosides and their Pd(II) complexes, Bioinorg Chem. 5, 353-358.

Norman, R.E., Ranford, J.D., Sadler, P.J. (1992) Studies of platinum(II) methionine complexes: metabolites of cisplatin. Inorg Chem 31, 877.

Pal, S. Das, D. Chattopadhyay, P., Sinha, C., Panneerselvam, K. and Lu, T.H. (2000) Spectral and structural studies of metal complexes of isatin 3-hexamethyleneiminylthiosemicarbazone prepared electrochemically. Polyhedron 19, 1263.

Paul, A.K., Mansuri-Torshizi, H., Srivastava, T.S., Chavan, S.J., Chitnis, M.P. (1993) Some potential antitumor 2,2'-dipyridylamine Pt(II)/Pd(II) complexes with amino acids: Their synthesis, spectroscopy, DNA binding, and cytotoxic studies. J Inorg Biochem. 50, 9.

Perrin, D.D. and Stunzi, H. in Leggett, D.J. (Ed.) (1985) Computational methods for the determination of formation constants. Plenum Press, New York, p.71.

Pettit, L., University of Leeds, personal communication, UK.

Pneumatikakis, G. (1984) 1:1 complexes of Pd(II) and Pt(II) with caeffine and their interaction with nucleosides. Inorg Chim Acta. 93, 5-11.

Pneumatikakis, G., Yannopoulos, A. Markopoulos and J. Angelopoulos, C. (1988) Monotheophylline and monotheophyllinato complexes of Pd(II) and their interactions with nucleosides. Inorg Chim Acta. 152, 101-106.

Puthraya, K.H., Srivastava, T.S., Amonkar, A.J., Adwankar, M.K., Chitnis, M.P. (1986) Some potential anticancer Pd(II) complexes of 2,2'-bipyridine with amino acids. J Inorg Biochem. 26, 45-54.

Rau, T., Shoukry, M.M., and van Eldik, R. (1997) Complex formation and ligand substitution reactions of (2-Picolylamine)palladium(II) with various biologically relevant ligands. Characterization of (2-picolylamine)(1,1-cyclobutanedicarboxylato)palladium(II). Inorg Chem. 36, 1454-1463.

Rauth, G.K., Pal, S., Das, D., Sinha, C., Slawin, A.M.Z., and Woollins, J.D. (2001) Synthesis, spectral characterization and electrochemical studies of mixed-ligand complexes of platinum(II) with 2-(arylazo)pyridines and catechols. Single-crystal X-ray structure of dichloro{2-(phenylazo) pyridine}platinum(II). Polyhedron. 20, 63.

Reedik, J. (1992) The relevance of hydrogen bonding in the mechanism of action of platinum antitumor compounds. Inorg Chim Acta. 198-200, 873-881.

Rees, D.C. (1980) Experimental evaluation of the effective dielectric constant of proteins. J Mol Biol. 141, 323-326.

Rogersa, N.K., Mooreb, G.R. and Sternberga M.J.E. (1985) Electrostatic interactions in globular proteins: calculation of the pH dependence of the redox potential of cytochrome C551. J Mol Biol. 182,613-616.

Rosenberg, B. (1980) In metal ions in biological systems, Sigel H, Ed., Dekker, New York 11,127-196.

Rosenberg, B. Van Camp, L. Trasko, J.E. and Mansour, V.H. (1969) Platinum compounds: a new class of potent antitumour agent. Nature. 222,385-386.

Roy, R., Chattopadhyay, P., Sinha, C. and Chattopadhyay, S. (1996) Synthesis, spectral and electrochemical studies of arylazopyridine complexes of palladium(II) with dioxolenes. Polyhedron. 15, 3361-3269.

Sabatini, A., Vacca, A. and Gans, P. (1992) Mathematical algorithms and computer programs for the determination of the equilibrium constants from potentiometric and spectrophotometric measurements. Coord Chem Rev. 120, 389-405.

Sabatini, A., Vacca, A. and Gans, P. (1974) Miniquad-A general computer programme for the computation of formation constants from potentiometric data. Talanta 21, 53-77.

Santra PK, Misra TK, Das D, Sinha C, Slawin AMZ, Woollins JD (1999), Chemistry of azopyrimidines. Part II. Synthesis, spectra, electrochemistry and X-ray crystal structures of isomeric dichloro bis[2-(arylazo)pyrimidine] complexes of ruthenium(II). Polyhedron. 18, 2869.

Shehata, M.R. (2001) Mixed ligand complexes of diaquo(2,2'-bipyridine)plladium(II) with cyclobutane-1,1-dicarboxylic acid and DNA constituents. Transition Met Chem. 26, 198-204.

Shehata, M.R., Shoukry, M.M., Abdel-Shakour, F.H. and van Eldik, R. (2009) Equilibrium studies on complex-formation reactions of Pd[(2-(2-aminoethyl)pyridine)$(H_2O)_2]^{2+}$ with ligands of biological significance and displacement reactions of DNA constituents. Eur J Inorg Chem.3912-3920.

Shehata, M.R., Shoukry, M.M., Nasr, F.M.H. and van Eldik, R. (2008) Complex-formation reactions of dicholoro(S-methyl-L-cysteine)palladium(II) with bio-relevant ligands. Labilization induced by S-donor chelates. Dalton Trans 779-786.

Shoukry, A., Rau, T., Shoukry, M., and van Eldik, R., (1998) Kinetics and mechanisms of the ligand substitution reactions of bis(amine)(cyclobutane-1,1-dicarboxylato) palladium(II). J Chem Soc Dalton Trans. 3105-3112.

Shoukry, M.M., Shehata, M.R., Abdel-Razik, A., Abdel-Karim, A.T. (1999) Equilibrium studies of mixed ligand complexes involving (1,2-diaminopropane)-Palladium(II) and Some bioligands. Monatsh Fur Chem. 130, 409-423.

Sigel, H. and Martin, R.B. (1982) Coordinating properties of the amide bond. Stability and structure of metal ion complexes of peptides and related ligands. Chem Rev 82, 385-426.

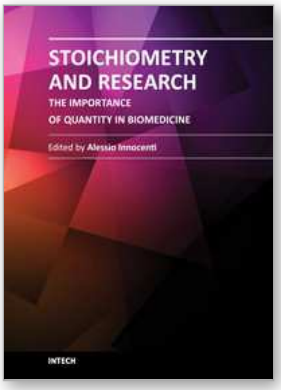
Sigel, H., Massoud, S.S., Corfu, N.A. (1994) Comparison of the extent of macrochelate formation in complexes of divalent metal ions with guanosine (GMP^{2-}), inosine (IMP^{2-}), and adenosine 5́-monophosphate (AMP^{2-}). The crucial role of N-7 basicity in metal ion-nucleic base recognition. J. Am. Chem. Soc. 116, 2958–2971

Tauler, R. Casassas, E. and Izquierdo-Ridorsa, A. (1991) Self-modelling curve resolution in studies of spectrometric titrations of multi-equilibria systems by factor analysis. Anal Chim Acta. 248, 447-458.

Tercero-Moreno, J.M., Matilla-Hernandez, A., Gonzalez-Garcia, S. and Niclos-Gutierrez, J. (1996) Hydrolytic species of the ion cis-diaqua(ethylenediamine) palladium(II) complex and of cis-dichloro(ethylenediamine) palladium(II): fitting its equilibrium models in aqueous media with or without chloride ion. Inorg Chim Acta. 253, 23.

Wong, E. and Giandomenico, C.M. (1999) Current Status of Platinum-Based Antitumor Drugs. Chem. Rev. 99, 2451.

Zekany, L. and Nagypal, I. (1985) in Leggett, D.J. (Ed.), Computational methods for the determination of formation constants. Plenum Press, New York, P. 71.



Stoichiometry and Research - The Importance of Quantity in Biomedicine

Edited by Dr Alessio Innocenti

ISBN 978-953-51-0198-7
Hard cover, 376 pages
Publisher InTech
Published online 07, March, 2012
Published in print edition March, 2012

The aim of this book is to provide an overview of the importance of stoichiometry in the biomedical field. It proposes a collection of selected research articles and reviews which provide up-to-date information related to stoichiometry at various levels. The first section deals with host-guest chemistry, focusing on selected calixarenes, cyclodextrins and crown ethers derivatives. In the second and third sections the book presents some issues concerning stoichiometry of metal complexes and lipids and polymers architecture. The fourth section aims to clarify the role of stoichiometry in the determination of protein interactions, while in the fifth section some selected experimental techniques applied to specific systems are introduced. The last section of the book is an attempt at showing some interesting connections between biomedicine and the environment, introducing the concept of biological stoichiometry. On this basis, the present volume would definitely be an ideal source of scientific information to researchers and scientists involved in biomedicine, biochemistry and other areas involving stoichiometry evaluation.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ahmed A. El-Sherif (2012). Coordination Chemistry of Palladium(II) Ternary Complexes with Relevant Biomolecules, Stoichiometry and Research - The Importance of Quantity in Biomedicine, Dr Alessio Innocenti (Ed.), ISBN: 978-953-51-0198-7, InTech, Available from: http://www.intechopen.com/books/stoichiometry-and-research-the-importance-of-quantity-in-biomedicine/coordination-chemistry-of-palladium-ii-ternary-complexes-with-relevant-biomolecules

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821