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Complexes of platinum(II) and palladium(II) with aminomethylphosphonic acid and glycylaminomethylphosphonic acid‡

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The complexing properties of aminomethylphosphonic acid, Gly-(*P*), and glycylaminomethylphosphonic acid, Gly-Gly-(*P*), with Pt^{II} and Pd^{II} were investigated pH-metrically at 25 °C and at an ionic strength of 0.1 mol dm^{-3} (KNO_3) using 'out of cell' titrations. The stability constants calculated indicate formation of complexes with a metal : ligand molar ratio of 1 : 1 and 1 : 2. The species and their distribution determined pH-metrically were confirmed by ^{31}P and ^{1}H NMR titrations. Comparison of our results for Gly-(*P*) and Gly-Gly-(*P*) with those for glycine and glycylglycine and other common dipeptides shows that the complexing properties of Pd^{II} and Pt^{II} towards both types are very similar.

The chemistry of cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, and its relatives has been widely investigated and reviewed, *e.g.* ref. 1. A few years ago, a class of antitumor-active complexes, containing phosphonic ligands, was reported[2,3] and the interactions of a variety of platinum phosphonato complexes, such as *cis*-diammine{nitrilotris(methylphosphonato)(2−)-*O*,*N*}platinum(II) and {(*R*,*S*)-cyclohexane-1,2-diamine}[nitrilotris(methylphosphonato)(2−)-*O*,*N*]platinum(II) with nucleotides were investigated using ^{1}H and ^{31}P NMR spectroscopy. In addition, other complexes of aminoalkylphosphonic acids with Pt^{II} and Pd^{II} were studied using NMR spectroscopy. The palladium(II) ions were often used instead of Pt^{II} because of their similar chemistry and faster kinetics.

The reactions of aminomethylphosphonic, Gly-(*P*), amino(phenyl)methylphosphonic, 1-aminoethylphosphonic, Ala-(*P*), and 1-(1-propylamino)ethylphosphonic, PrAla-(*P*), acids with $K_2[PdCl_4]$ were investigated by Matczak-Jon and Wojciechowski[4] using ^{1}H, ^{13}C and ^{31}P NMR spectroscopy. The aminoalkylphosphonic acids formed {N,O} chelates of the $[PdA_2]^{2-}$ and $[PdAX_2]^{2-}$ types, where $X = Cl^-$, H_2O or OH^-. Co-ordination only *via* amine was observed in strongly alkaline solution, except for PrAla-(*P*). On the other hand, co-ordination only *via* the phosphonic group, which was found in the systems with platinum(II) ions by Appleton *et al.*,[5] was not observed in these systems. This is probably caused by the faster kinetics of the reaction of Pd^{II} compared to Pt^{II}. Matczak-Jon and Wojciechowski[6] also studied the ^{13}C and ^{31}P NMR spectra of palladium(II) systems with 3-amino-3-phosphonopropionic, α-Asp-(*P*), and 2-amino-3-phosphonopropionic, β-Asp-(*P*), acids: $\{N,O_P\}$ chelates were found in the system with α-Asp-(*P*) in a wide pH region, and in the alkali region for the β-Asp-(*P*) system; in the acid region β-Asp-(*P*) formed $\{N,O_C\}$ chelates.

Glowacki *et al.*[7] used the ^{31}P NMR spectroscopy of palladium(II) complexes with 1-aminoalkylphosphonic acids to determine the enantiomeric purity of the acids. In D_2O solution at a pD of about 9 stable chelating pairs of the $[PdA_2]^{2-}$ type were formed. The ^{31}P NMR spectra of these complexes exhibited different signals for the *S*,*R* and chiral (*S*,*S*) or (*R*,*R*) forms of the co-ordinated ligand for most acids. In addition, the authors investigated the diastereoselective complexation of amino(phenyl)methylphosphonic acid [mixture of the racemate and the (−) form of the acid in a ratio of 1 : 1] with palladium(II) ions. This reaction was found to be non-diastereoselective because the difference from the theoretical abundance of the diastereoisomers was only 0.5%.

Appleton *et al.*[5] used ^{15}N, ^{31}P and ^{195}Pt NMR spectroscopy to investigate the reactions of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and *cis*-$[Pt(NH_3)_2(OH)_2]$ with aminoalkylphosphonic acids of the $NH_2(CH_2)_nPO_3H_2$ type [$n = 1$, Gly-(*P*); $n = 2$, β-Ala-(*P*); $n = 3$, gaba-(*P*)]. In the acidic region at pH from 1.5 to 4 a complex with the acid bonded only *via* the oxygen atoms was found. At pH above 4 a complex with the acid and hydroxy group bridging $Pt(NH_3)_2^{2+}$ species formed together with $[Pt(NH_3)_2\{Gly\text{-}(P)\}]$ and $[Pt(NH_3)_2\{\mu\text{-}Gly\text{-}(P)\}Pt(NH_3)_2(H_2O)]^{2+}$. In the alkaline region at pH of about 12.5, $[Pt(NH_3)_2(OH)_2]$ reacted with Gly-(*P*) during 1 week, in contrast to glycine (Gly), and formed *cis*-$[Pt(NH_3)_2\{Gly\text{-}(P)\}_2]^{2-}$, where the Gly-(*P*) was bonded only through the amine groups. Under the same conditions, β-Ala-(*P*) formed a chelate and gaba-(*P*) did not react.

Complexes of phosphonodipeptides with the soft metals have not been reported, except in our preliminary results[8] and a study of the complexing properties of (*S*,*S*) and (*S*,*R*) diastereoisomers of 1-(methionylamino)ethylphosphonic acid, Met-Ala-(*P*).[9] The aim of the present paper was to study the complexing ability of the simplest aminoalkylphosphonic acid, *i.e.* aminomethylphosphonic acid $H_2NCH_2PO_3H_2$, Gly-(*P*), and the simplest phosphonodipeptide, *i.e.* glycylaminomethylphosphonic acid $H_2NCH_2CONHCH_2PO_3H_2$, Gly-Gly-(*P*), with Pt^{II} and Pd^{II} using potentiometry and NMR spectroscopy. This combination of methods should make it possible to find the complexes formed, their means of co-ordination and their distribution in solution and thus to gain an understanding of the co-ordination ability of the soft metals towards biological materials.

Results

Potentiometry

The complexes of Pt^{II} and Pd^{II} are kinetically stable and, therefore, an 'out of cell' titration procedure had to be used. The UV spectra for the palladium(II) systems and pH values for platinum(II) systems indicated that the former were in equilibrium after 1 day, the latter after 10 d, similar to the system with Met-Ala-(*P*).[9] The dependence of absorbance on time (first 180 min) for the Pd^{II}–Gly-Gly-(*P*) system and dependence of $-\log [H^+]$ on time (first 7 d) for the Pt^{II}–Gly-(*P*) system are shown in Figs. 1 and 2. They indicate that in the acidic region the equilibrium was usually attained faster, but in the alkaline region it was necessary to wait for the period of time mentioned above.

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‡ Dedicated to Professor Jaraslav Podlaha, who focused our attention on co-ordination chemistry of the organophosphorus ligands, on the occasion of his 60th birthday.

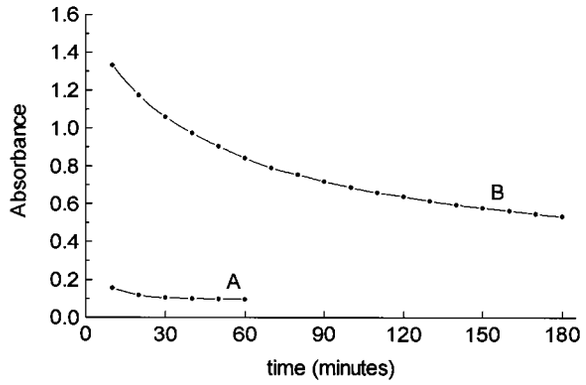

Fig. 1 Dependence of absorbance on time in the system Pd^{II}–Gly-Gly-(*P*), $c_{Pd} = 0.0015$ mol dm^{-3} (M : H_2A = 1 : 2), $\lambda = 305$ nm, pH after 3 d: A, pH 4.2; B, pH 9.1

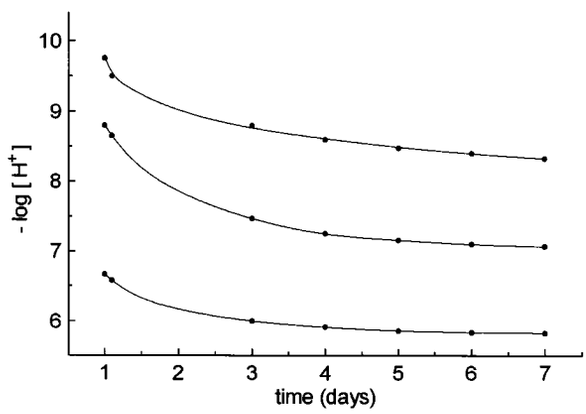

Fig. 2 Dependence of pH on time in the system Pt^{II}-Gly–(*P*), $c_{Pt} = 0.005$ mol dm^{-3} (M : H_2A = 1 : 2)

Determination of the complex formation constants is rather complicated due to documented instability of $[Pt(H_2O)_4]^{2+}$ and $[Pd(H_2O)_4]^{2+}$ ions in aqueous solutions. Therefore, we used the chloro-complexes $K_2[PdCl_4]$ and $K_2[PtCl_4]$ as sources of Pd^{II} and Pt^{II} and the individual complex formation constants of the chloro-complexes were included in the calculation of the stability constants with Gly-(*P*) and Gly-Gly-(*P*). Several papers have dealt with the complex formation constants of the chloro-complexes. Owing to the ionic strength employed, we used the constants log K_{1-4} 4.94, 4.0, 2.92, 2.1 given by Elding[10] for the platinum(II) systems and log K_{1-4} 4.58, 3.55, 2.42, 1.13 according to Kragten[11] for the palladium(II) systems, as in our parallel paper.[9] Therefore, the accuracy of the stability formation constants determined in the systems studied is affected by the correctness of the complex formation constant values for the chloro-complexes. The accuracy of the constants and chemical models of the systems studied could be also affected by formation of chlorohydroxo complexes. However, in the literature, we have only been able to find constants for the reactions (1)–(4)

$$[Pt(OH)_4]^{2-} + Cl^- \rightleftharpoons [Pt(OH)_3Cl]^{2-} + OH^-; \quad \log K = 10.48 \quad (1)$$

$$[Pt(OH)_3Cl]^{2-} + Cl^- \rightleftharpoons [Pt(OH)_2Cl_2]^{2-} + OH^-; \quad \log K = 10.00 \quad (2)$$

$$[Pt(OH)_2Cl_2]^{2-} + Cl^- \rightleftharpoons [Pt(OH)Cl_3]^{2-} + OH^-; \quad \log K = 9.52 \quad (3)$$

$$[Pt(OH)Cl_3]^{2-} + Cl^- \rightleftharpoons [PtCl_4]^{2-} + OH^-; \log K = 8.66 \quad (4)$$

of Pt^{II}.[12] Whether these values were or not involved in the calculation of the formation stability constants of Pt^{II} with Gly-(*P*) or Gly-Gly-(*P*) virtually the same values were obtained for a region of pH 2–9. If the literature log *K* values for the hydroxo-chloro complexes were altered in the range ±50% the same values were again obtained in the range of pH. We assume that the hydroxochloro complexes do not significantly influence the systems investigated and therefore we do not include equilibria (1)–(4) in our considerations.

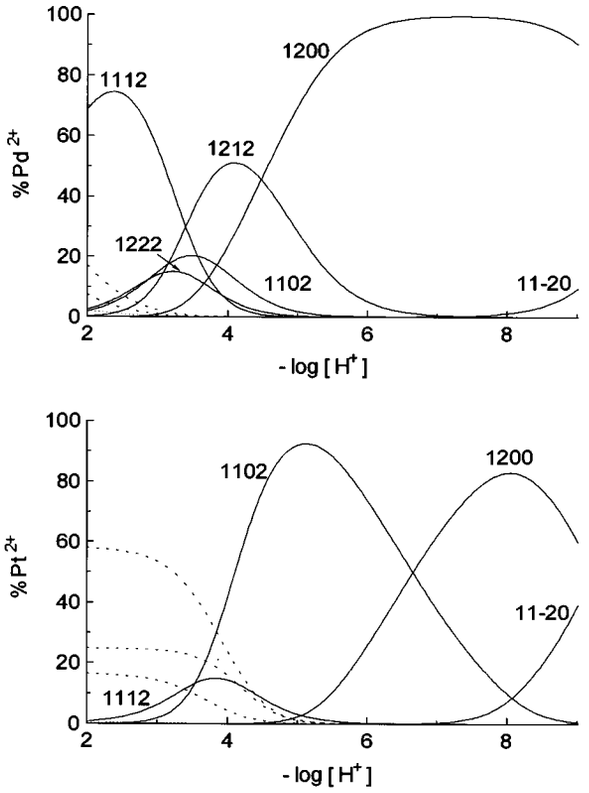

Fig. 3 Distribution diagrams of complexes formed in the Pd^{II}–Gly-(*P*) (upper) and Pt^{II}–Gly-(*P*) (lower) systems as a function of $-\log[H^+]$ ($c_M = 0.005$, $c_{H_2A} = 0.01$ mol dm^{-3}). The dashed curves belong to chloro-complexes. Numbering of the species corresponds to the molar ratio M : A : H : Cl

The protonation constants determined for Gly-(*P*)[13,14] and Gly-Gly-(*P*)[15] are in good agreement with those published previously. The stability constants for formation of the complex of $[PdCl_4]^{2-}$ or $[PtCl_4]^{2-}$ with Gly-(*P*) are listed in Table 1 and distribution diagrams are shown in Fig. 3. The Experimental section gives the procedure for titrations of solutions with a metal to ligand molar ratio of 1 : 2 and 1 : 4. However, when the titration data from both the ratios were calculated together the distributions found did not correspond to the abundance of the species observed in the ^{31}P NMR spectra above pH 9 in contrast to the other species (see below), probably because of the poor reproducibility of the titration in the alkaline region and/or the formation of the hydroxo-species. For this reason, the titrations with molar ratios of 1 : 2 and 1 : 4 were only calculated in the region of pH 2–9. From the distribution diagrams and from Table 1 it can be seen that the palladium(II) and platinum(II) systems are similar, but not identical because of the different stabilities of the chloro-complexes. Thus, Pd^{II} begins to form complexes with Gly-(*P*) at a pH of about 1 and Pt^{II} forms the same complexes at a pH of about 3. Both the systems contain the protonated species 1112, *i.e.* $[M(HA)Cl_2]^-$ with the {N,O} co-ordinated ligand which deprotonates in a higher pH region and forms 1102, *i.e.* $[MACl_2]^{2-}$ (H_2A = aminoalkylphosphonic acid). The constant $pK_{MAH^{-H}}$ was derived for process (5). The log

$$[M(HA)Cl_2]^+ \longrightarrow [MACl_2] + H^+; \; pK_{MAH^{-H}} = \log \beta_{1112} - \log \beta_{1102} \quad (5)$$

Table 1 Logarithmic protonation constants of Gly-(*P*) and Gly-Gly-(*P*) and stability constants for their complex formation with Pt^{II} and Pd^{II} at 25 °C and $I = 0.1$ mol dm^{-3}

M^{2+}	A^{2-}	H^+	Cl^-	Gly-(*P*)			Gly-Gly-(*P*)	
0	1	1	0	10.02(1)			8.18(1)	
0	1	2	0	15.42(1)			14.30(1)	
0	1	3	0	15.85(4)			15.51(2)	
				Pd^{II}	Pt^{II}	$Pt^{II\,a}$	Pd^{II}	$Pt^{II\,b}$
1	2	2	2	38.76(5)	—	—	—	36.07(3)
1	2	1	2	35.68(2)	—	—	—	—
1	2	0	0	27.51(2)	21.62(4)	24.06(2)	26.27(6)	20.40(2)
1	2	−2	0	—	—	2.87(20)	10.99(8)	3.58(11)
1	1	1	2	24.65(2)	22.99(8)	23.70(5)	—	—
1	1	0	2	21.08(6)	19.45(2)	20.11(1)	—	—
1	1	0	1	—	—	—	20.54(5)	16.85(7)
1	1	−1	1	—	—	—	16.74(6)	10.35(23)
1	1	−2	0	4.73(5)	−0.08(8)	2.19(3)	8.67(7)	1.88(2)
1	1	−3	0	—	—	—	−1.51(8) [b]	−7.59(5)

[a] Concentration of Pt^{II}, $c_{Pt} = 0.15$; concentration of Gly-(*P*), $c_{H_2A} = 0.3$ mol dm^{-3}. [b] Orientative values (see text).

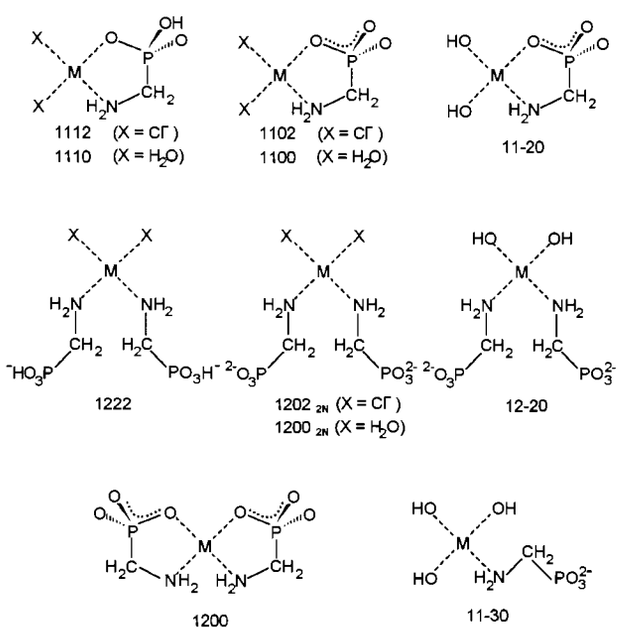

Fig. 4 Tentative structures of the species found in the platinum(II) and palladium(II) systems with Gly-(*P*). Only *cis* isomers are shown; the probability of the *trans* isomer is the same

$K_{MAH^{-1}}$ values 3.57 for the palladium(II) and 3.54 for platinum(II) systems are virtually the same and correspond to a decrease in the pK_2 of the phosphonic group. Although only the concentration of H^+ was measured and the number of Cl^- ions in the species is not known, we observed the best results, *i.e.* the least deviations and the best statistical parameters for the 1102 stoichiometry, namely $[MACl_2]^{2-}$. However, it is clear from the NMR spectra (see below) that the formation stability constants of $[MACl_2]^{2-}$ also include a small amount of the $[MACl(H_2O)]^-$ and $[MA(H_2O)_2]$ species. Deprotonation of co-ordinated water continues in the region above about pH 8, and formation of the 11–20 species, *i.e.* $[MA(OH)_2]^{2-}$, starts as well as substitution of chlorides.

In addition to complexes with a molar ratio of 1:1, 1:2 complexes are formed in both systems. In the platinum(II) system the protonated 1220 $[Pt(HA)_2]$ complexes begin to form in the pH region around 3.5 in an amount of less than 5%, confirmed by the NMR spectra, and thus these species have not been included in the 'potentiometric' model. Complex 1200, *e.g.* $[PtA_2]^{2-}$, is predominant in the neutral region. In the palladium(II) system the protonated 1222 species $[Pd(HA)_2Cl_2]^{2-}$ exists at a pH of about 2 and 1212 $[PdA(HA)Cl_2]^{3-}$ at a pH of about 4. The predominant species in the neutral pH region is again 1200, *i.e.* $[PdA_2]^{2-}$. The value of $\log(\beta_{1200}/\beta_{1102})$ for the palladium(II) system, 6.46, is higher than 2.73 for the platinum(II) system because of the higher stability of the $[MACl_2]^{2-}$ complex with Pt^{II}. The assumed structures are depicted in Fig. 4.

The potentiometric titration of the platinum(II) system with Gly-(*P*) at the same concentration as for the NMR measurements was also carried out in view of the fact that the chemical model and distribution of the species found were verified by NMR titrations. The constants found are also listed in Table 1. It is clear that the same species were determined; however, the stability constants at a ligand concentration of 0.3 mol dm^{-3} are slighly higher than at a concentration of 0.01 mol dm^{-3}.

The stability constants for formation of the complexes of both the metals with Gly-Gly-(*P*) are listed in Table 1 and the distribution diagrams are shown in Fig. 5. It is clear that Pd^{II} forms complexes with Gly-Gly-(*P*) in metal:ligand molar ratios of 1:1 and 1:2. The 1:1 complexes start to form 1101 species, *i.e.* $[PdA(Cl)]^-$, in the acidic region. These correspond to the structural motif known from common dipeptides[16] with co-ordinated amine, peptide amide, phosphonate groups and chloride as shown in Fig. 6. The NMR spectra confirmed the formation of a chelate *via* the peptide amide and phosphonic groups and thus the phosphonic group is protonated. In next step in the region of pH 3–7 the 1101 species deprotonates and forms 11–11, *i.e.* $[PdAH_{-1}Cl]^{2-}$. The constant pK_1^{-1} derived for process (6) would correspond to the pK_2 of the free

$$[MA(Cl)]^- \longrightarrow [MAH_{-1}Cl]^{2-} + H^+;\ pK_1^{-1} = \log \beta_{1101} - \log \beta_{11\text{-}11} \quad (6)$$

phosphonate. The decrease to a value of 3.80 is close to the values observed for systems with Gly-(*P*). In the alkaline region substitution of chloride by the hydroxy group or deprotonation of the co-ordinated water molecule leads to the formation of the 11–20 species and, in the strongly alkaline region, to substitution of the phosphonic group by hydroxide leads to the 11–30 species. The value of the derived constant $pK_1^{-2} = 8.07$ [equation (7)] corresponds to the values found for

$$[MAH_{-1}Cl]^- \longrightarrow [MAH_{-2}]^{2-} + H^+ + Cl^-;\ pK_1^{-2} = \log \beta_{11\text{–}11} - \log \beta_{11\text{–}20} \quad (7)$$

analogous systems with Cu^{II}.[15] Complexes with a molar ratio of 1:2 prefer co-ordination only *via* the amine and peptide amide groups (see NMR section) and the 1200 and 12–20 species indicate the presence of protonated and deprotonated phosphonic groups as is shown in Fig. 6.

The results for the system with Pt^{II} are only orientative due to partial (less than 10%) hydrolysis of the peptide bond as is discussed in the NMR part. Nevertheless, it is clear from the distribution diagram that the formation of the complexes is shifted to a pH of about 3 due to the higher stability of the chloro complexes as in the Gly-(*P*) systems. Comparison with the palladium(II) systems also indicates that Pt^{II} prefers species with a molar ratio of 1:2. The predominant species in the acid and neutral regions are complexes 1222 and 1200 after deprotonation of the phosphonic groups. Species 11–20 and 11–30 with a metal:ligand molar ratio of 1:1 were found only in the alkaline region following formation of the hydroxo-complexes.

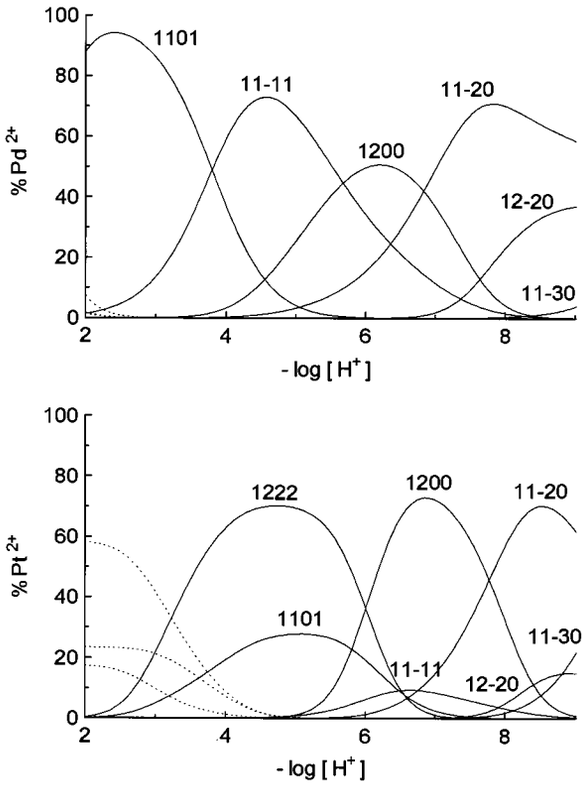

Fig. 5 Distribution diagrams of complexes formed in the Pd^{II}–Gly-Gly-(*P*) (upper) and Pt^{II}–Gly-Gly-(*P*) (lower) systems as a function of $-\log[H^+]$ ($c_M = 0.005$, $c_{H_2A} = 0.01$ mol dm^{-3}). The dashed curves belong to chloro-complexes. Numbering of the species corresponds to the molar ratio M : A : H : Cl

NMR titrations

To verify the presence of the species found potentiometrically, NMR titration of the systems equilibrated was carried out. The δ_P *vs.* pD and δ_H *vs.* pD plots for the systems studied are shown in Figs. 7–9. The open circles denote very small peaks which correspond to species with very low abundance (estimated to be less than 5%). The δ_P *vs.* pD plot contains two regions of δ_P: one between 10 and 20 ppm corresponding to the non-co-ordinated phosphonic group similar to curves L [free Gly-(*P*) or Gly-Gly-(*P*)] and another one above 40 ppm that, according to previous results,[2b,5,17] corresponds to the formation of a five-membered chelate *via* the amine or amide and phosphonic groups. Changes in δ_P with pD correspond to the deprotonation of the phosphonic group similar to the changes observed for the free phosphonates.

Thus, if we take into consideration the abundance of the species determined potentiometrically for the system Pt^{II}–Gly-(*P*) and the results of Matczak-Jon and Wojciechowski[4] then curve A should correspond to the 1112 form and the changes in its chemical shift in the region pD 4–6 correspond to deprotonation and formation of the 1102 species. The structures of the species assumed to be present are depicted in Fig. 4. In both species Gly-(*P*) is bonded as a chelate with a protonated or deprotonated phosphonic group and two chlorides occupy the

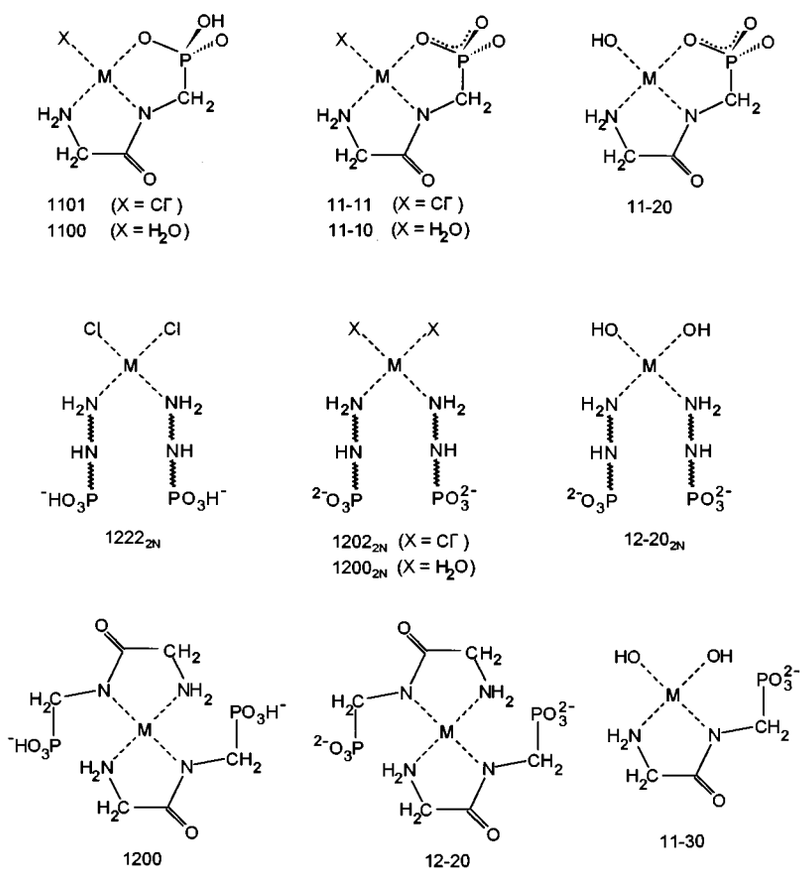

Fig. 6 Tentative structures of the species found in the platinum(II) and palladium(II) systems with Gly-Gly-(*P*). Only *cis* isomers are shown; the probability of the *trans* isomer is the same

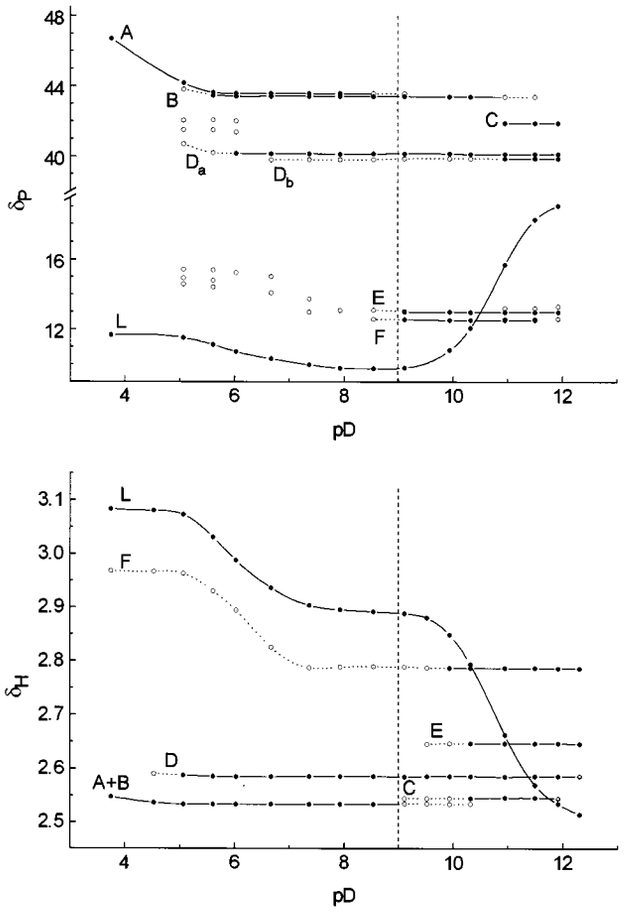

Fig. 7 Variation of δ_P *vs.* pD (upper) and δ_H (lower) for the Pt^{II}–Gly-(*P*) system. Open circles indicate species of low relative abundance, the broken vertical line the region of pH used for data treatment. Curve A corresponds to deprotonation of phosphonic group in species 1112 and formation of 1102, B to 1100, C to 11–20, D to 1200 (D_a, D_b = probable *cis*, *trans* isomers), E to 11–30, F to species co-ordinated only through the amine group, *i.e.* 1222_{2N}, 1202_{2N}, 1200_{2N} and $12\text{-}20_{2N}$, and A to free Gly-(*P*)

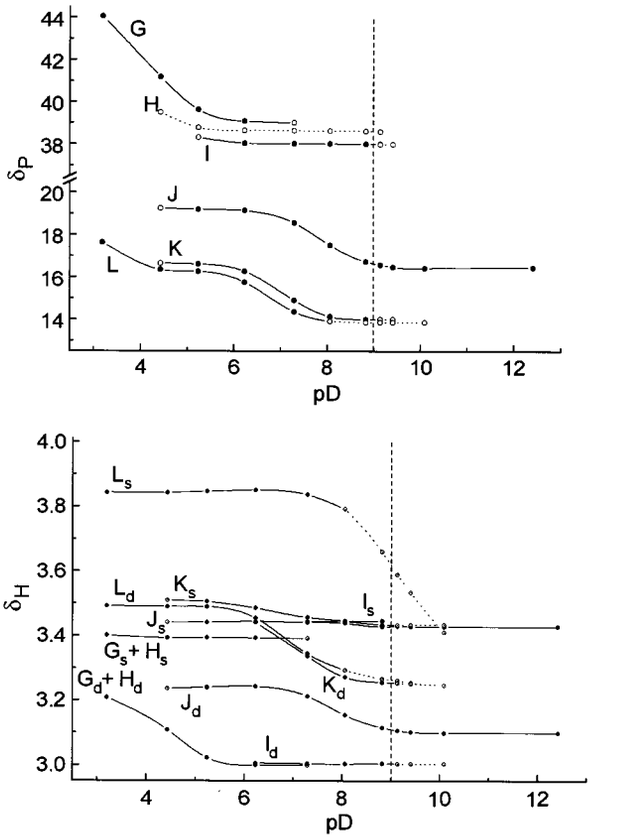

Fig. 8 Variation of δ_P *vs.* pD (upper) and δ_H (lower) for the Pd^{II}–Gly-Gly-(*P*) system. Key as in Fig. 7. Curves G corresponds to deprotonation of the phosphonic group in species 1101 and formation of 11–11, H (low abundance) to 11–10, I to 11–20, J to deprotonation of phosphonic groups in 1200 and formation of 12–20, K to species co-ordinated only through the amine group, *i.e.* 1222_{2N}, 1200_{2N} and $12\text{–}20_{2N}$ and A to free Gly-Gly-(*P*). Subscripts s and d correspond to the methylene in Gly and Gly-(*P*) parts of the molecule

remaining two positions. Virtually the same chemical shift was found by Appleton *et al.*[5] at pH 7.0, $\delta_P = 42.75$, and deprotonation of the phosphonic group of $[Pt(NH_3)_2\{HGly\text{-}(P)\text{-}N,O\}]^+$ was observed at about pH 3. Substitution of the chlorides by water would not significantly influence δ_P. Therefore, curve B which is very close to A should correspond to the 1100 species. The hydrogen atoms of methylene would not be influenced by substitution on Pt^{II} and, in the δ_H *vs.* pD plot, only one curve A + B corresponds to the 1112, 1102 and 1100 secies. Consequently, points C in both the δ_P and δ_H *vs.* pD plots that are observed only in the alkaline region correspond to deprotonation of bonded water molecules and formation of the hydroxo-complex 11–20 found potentiometrically. There are two sets of points denoted as D in the δ_P region around 40 ppm that are very close and should correspond to the 1200 species. This complex contains two molecules of Gly-(*P*) and thus can form *cis* and *trans* isomers. The small differences in δ_P correspond to differences in the positions of the phosphonic groups in the *cis* isomer in contrast to the *trans* isomer. The methylene protons would not be influenced by *cis* and *trans* isomerisation and, thus, in the δ_H *vs.* pD plot, only one set of the points was observed for the 1200 species. In addition to free Gly-(*P*), two sets of points E and F were observed in the δ_P *vs.* pD plot, corresponding to the region of the unco-ordinated phosphonic group. Points F reflect the 1222_{2N}, 1202_{2N}, 1200_{2N} and $12\text{–}20_{2N}$ species in which two molecules of Gly-(*P*) are bonded to the metal only through the amine group as is shown in Fig. 4. The 1222_{2N}, 1202_{2N} and 1200_{2N} species are present in very small

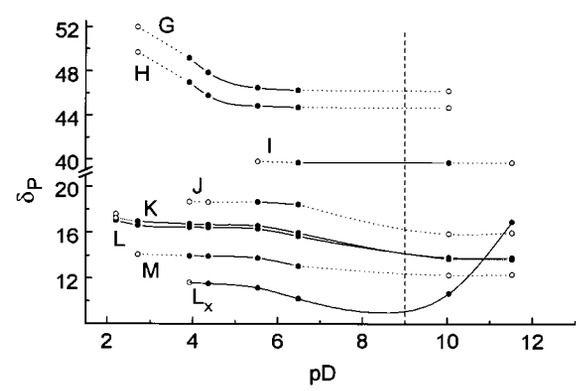

Fig. 9 Variation of δ_P *vs.* pD for the Pt^{II}–Gly-Gly-(*P*) system. Empty circles indicate species of low relative abundance. Curves G belong to deprotonation of the phosphonic group in species 1101 and formation of 11–11, H to 11–10, I to 11–20, J to deprotonation of phosphonic groups in 1200 and formation of 12–20, K to species co-ordinated only through the amine group, *i.e.* 1222_{2N}, 1200_{2N} and $12\text{–}20_{2N}$, A to free Gly-Gly-(*P*), L_x to Gly-(*P*) after hydrolysis of the peptide bond and M to a complex of Pt^{II} with Gly-(*P*)

amounts (less than 5%) and were not determined potentiometrically. Changes in both δ_P and δ_H correspond to deprotonation of the phosphonic group. Points E indicate the presence of the 11–30 species which contains three hydroxo-groups as is shown in Fig. 4. In the part on potentiometric titrations we mentioned the poor reproducibility in the alkaline region and the influence of chlorohydroxo complexes. Therefore, the stability constants of the hydroxo-complexes could not be determined from the calculation.

The NMR titration of the Pd^{II}–Gly-(*P*) system was studied previously by Matzcak-Jon and Wojciechowski[4] and the results correspond to our potentiometric titration, our orientative NMR measurement and are related to the results from the platinum(II) system; therefore we did not investigate the Pd^{II}–Gly-(*P*) system using NMR spectroscopy.

The δ_P *vs.* pD and δ_H *vs.* pD plots for the Pd^{II}–Gly-Gly-(*P*) system are depicted in Fig. 8 and the δ_P *vs.* pD for Pt^{II}–Gly-Gly-(*P*) system in Fig. 9. The structures of the species assumed are in Fig. 6. As in the Pt^{II}–Gly-(*P*) system, the δ_P region around 40 ppm corresponds to the formation of a chelate and co-ordination of both the phosphonic and peptide amide groups. The region corresponding to free Gly-Gly-(*P*) around 18 ppm corresponds to the unco-ordinated phosphonic group. In the ^1H NMR spectra we can distinguish two different methylene groups, one in the Gly-(*P*) part, split by phosphorus and denoted by subscript d, and the other in the Gly part denoted by subscript s. Points G should correspond to the 1101 species with co-ordinated amine, amide and phosphonic groups, which is also protonated. The remaining co-ordination site is probably occupied by chloride as was found by potentiometry. The changes in δ_P in the region pD 3–6 reflect deprotonation of the phosphonic group and formation of the 11–11 species. The deprotonation is noticeable in δ_H changes of the G_d points for the methylene group in the Gly-(*P*) part of molecule. The Gly part is not influenced by deprotonation. As in the Gly-(*P*)–Pd^{II} system, substitution of chloride by a molecule of water yields 11–10, *i.e.* points H which lie near points G. However, the intensity of the H peaks is very low, less than 5%, and therefore this type of species has not been included in the 'potentiometric' model. A similar species with tridentate co-ordinated Gly-Gly-(*P*) and a hydroxide in the remaining position corresponds to 11–20 with high abundance in the potentiometrically determined distribution diagrams and points I in the δ_P *vs.* pD and δ_H *vs.* pD plots. There are three curves J, K, L in the δ_P *vs.* pD plot corresponding to the non-co-ordinated phosphonic group in the palladium(II) system. Curve L corresponds to the titration curve of the free dipeptide. The J curve corresponds to the 1200 species and its deprotonation and formation yield 12–20. Deprotonation of phosphonic groups was reflected in changes of δ_H for methylene in the Gly-(*P*) part of molecule. The K points with relatively high intensity correspond to species with the same metal : ligand molar ratio, 1222, 1202, 1200 and 12–20, in which the dipeptide is co-ordinated only through the amine group as is shown in Fig. 6. The difference in co-ordination could not be distinguished by potentiometry. Deprotonation of phosphonic groups in curve K occurs in the same region as for L for the free dipeptide.

The same species were observed in the platinum(II) system, and in addition, other sets of points M and L_x. If we compare the L_x curve with the L curve for Gly-(*P*) in Fig. 7 we can see that both are virtually the same and in the same region of δ_P. This presence of Gly-(*P*) in the Gly-Gly-(*P*) system indicates hydrolysis of the peptide bond. The M points should correspond to Gly-(*P*) co-ordinated *via* the amine group. The hydrolysis was observed in 0.01 mol dm^{-3} solution after 24 h. On the other hand, the concentration of Gly-(*P*) in the solution seems to be less than 10%. From this point of view the values of the stability constants for the Pt^{II}–Gly-Gly-(*P*) system can only be orientative.

Discussion

The equilibria in the palladium(II) systems were checked by UV/VIS spectroscopy (Fig. 1). Changes in the spectra were observed in the acidic region up to 60 min and in the alkaline region over 180 min. Therefore, titrations were carried out after 3 d when we expected that the system had reached equilibrium. The titration procedure for the palladium(II) systems is often the same as that for common metals or the time of titration is extended *e.g.* to 27 h in the work of Ganadu and co-workers.[18] We have found that such extension is inappropriate because the liquid-junction potential of the glass electrode is not constant for such a long time. Therefore, it is necessary to use the 'out of cell' method for palladium(II) as well as for platinum(II) systems.

The establishment of equilibrium in the platinum(II) systems was checked by potentiometry and some small changes in pH values were observed even after 6 d. Titrations in these systems were usually carried out after 10–12 d. Appleton *et al.*[5] observed that a system of $[Pt(NH_3)_2(OH)_2]$ with Gly-(*P*) did reach equilibrium in the alkaline region even after several weeks. Probably, the changes in pH values with time in the strongly alkaline region are small and, therefore, this method is not as convenient for checking equilibria as is the NMR method used by Appleton.[5] The poor reproducibility in the region above pH 9 was probably due to lack of attainment of equilibrium. Owing to this fact and an influence of the chlorohydroxo complexes in the alkaline region, the formation constants were calculated only from region of pH 2–9.

Palladium(II) and especially Pt^{II} forms hydroxo-complexes and μ-OH complexes in aqueous solution very easily. The complex cisplatin forms hydroxo-complexes at neutral pH.[19] To avoid the formation of these complexes and consequently partial reduction of Pt^{II} or Pd^{II} to the metals we used an excess of phosphonic acid, the metal : phosphonic acid molar ratio being 1 : 2 and 1 : 4 for both acids, and a sample preparation involving addition of solutions of $K_2[PtCl_4]$ or $K_2[PdCl_4]$ to a solution of the phosphonic acid that had already been neutralised to the pH estimated.

A sample of the Pt^{II}–Gly-(*P*) system at pD 6.24 and a molar ratio M : A = 1 : 2 was checked by ^{195}Pt NMR spectroscopy. Two peaks at δ −1610 and −1618 were observed corresponding to two major peaks at δ 43.0 and 39.5 in the ^{31}P NMR spectrum of the same sample and assigned to species 1102 and 1200, *i.e.* $[PtACl_2]^{2-}$ and $[PtA_2]^{2-}$. No signals that would correspond to the hydroxo species in the range from δ −1185 (aquatrichloro complex)[20] or −1161 $\{[Pt(NH_3)_2(OH)]_2\}$[21] to −1572 $\{[Pt(NH_3)_2(OH)_2]\}$[21] were observed.

The formation of hydroxo-complexes 11–20 in both the platinum(II) and palladium(II) systems with Gly-(*P*) starts at pH > 8 and with Gly-Gly-(*P*) at pH ≈6. The region of pH for deprotonation of co-ordinated H_2O corresponds to that found recently for $[Pt(en)(H_2O)_2]^{2+}$ (en = ethane-1,2-diamine).[22] Our experience points to the fact that the use of hydroxo complexes as starting materials is inconvenient due to slow kinetics of substitution. The chloro complexes react much faster and formation of the chlorohydroxo complexes in the range of pH 2–9 is negligible for the systems studied.

The structures assumed for the species determined potentiometrically are depicted in Figs. 4 and 6. Some of the species can exist as *cis* or *trans* isomers, however in the figures only *cis* isomers are shown even though the probability of the *trans* isomer is the same. Using this method we cannot distinguish between the isomers or their mixture. From NMR spectra we could only suggest that the abundance of one isomer was higher than that of the other one.

A comparison of the chemical models determined potentiometrically and by NMR spectroscopy for the systems investigated indicates good agreement. The distribution diagrams also correspond to the intensities of the ^1H and ^{31}P-$\{^1H\}$ NMR peaks of the corresponding species. It is clear that the species found potentiometrically can include several species indicated by NMR spectroscopy, *e.g.* substitution of chloride by water in the co-ordination sphere, *cis-trans* isomers or a different means of co-ordination, as was observed for 1200 and 1200_N for $[Pt\{Gly\text{-}(P)\}_2]^{2-}$. On the other hand, one peak in the NMR spectrum can correspond to two species with different extents of protonation. However, the deprotonation of the phosphonic group is confirmed by the shape of the NMR titration curve.

As mentioned above, a NMR investigation of the Pd^{II}–Gly-(*P*) system was carried out by Matczak-Jon and Wojciechowski.[4] Our orientative NMR measurements confirmed the published results and our potentiometrically determined chemical model also corresponds to the species found by NMR spectroscopy, except for $[\{PdA(H_2O)(OH)\}_2]^{2-}$. Matczak-Jon and Wojciechowski[4] assigned this species to the peak with low intensity in the ^{31}P-{^1H} NMR spectrum in the alkaline region. However, it was not included in the potentiometric chemical model due to its low abundance and the high deviation of its stability constant.

A great many papers dealing with the complexing properties of Pt^{II} and Pd^{II} with Gly and other common amino acids have been published and the results reviewed, *e.g.* by Kozlowski and Pettit.[23] Determination of the stability constants is described in several papers; however only in two dealing with Gly the authors include the stability constants of the chloro-complexes in the calculation and chemical model. Anderegg and Malik[24] determined the stability constant for $[PdL_2]$ (L = glycinate) at I = 1 mol dm^{-3} ($NaClO_4$), log β_{12} = 27.50, and Yatsimirski[25] investigated the same system at I = 0.15 mol dm^{-3} (NaCl) and determined log β_{1102} = 20.08 and log β_{1200} = 26.84. The values of these stability constants are very close to those determined for the analogous species in the Pd^{II}–Gly-(*P*) system (Table 1). The species found in the Gly system are analogous to those determined in the system containing Gly-(*P*). We have so far been unable to find stability constant values for the system of Pt^{II} with Gly in the literature. Nevertheless, comparison of the palladium(II) systems with Gly-(*P*) and Gly indicates that the complexing properties of the two ligands towards soft metals would be analogous.

In addition, we have not been able to find any stability constant values for systems of the soft metals with Gly-Gly or other dipeptides, except for the system of $[Pd(en)(H_2O)_2]$ with Gly-Gly where pK = 3.76 (deprotonation and simultaneous co-ordination of the peptide amide bond).[26] On the other hand, a number of papers have dealt with the means of co-ordination of Gly-Gly or other dipeptides with Pd^{II} and Pt^{II}. Wilson and Martin[16] investigated the CD spectra of the systems and found co-ordination *via* amine-N, amide-N and carboxyl-O. The fourth co-ordination position was occupied by Cl^-, H_2O or in the alkaline region by OH^-. Analogously, the same means of co-ordination was observed by Watabe *et al.*[27] after reaction of $K_2[PtCl_4]$ with Gly-X or X-Gly where X = Gly, Ala, Val or Leu. Kozlowski *et al.*[28] investigated the influence of the metal : dipeptide molar ratio in systems of $K_2[PdCl_4]$ with Gly-L-Phe and L-Phe-Gly using ^1H NMR and UV/VIS spectroscopy. The results obtained for the 1:1 ratio confirmed the means of co-ordination through the amine, amide and carboxylic groups. For the 1:2 ratio in the alkaline region the formation of a species with two dipeptide molecules co-ordinated to the metal *via* the amine and peptide amine groups was observed. Appleton *et al.* studied the reactions of Gly-Gly with $[Pd(en)(H_2O)_2]$[29] and with *cis*-$[Pt(NH_3)_2(H_2O)_2]$.[30] From the dependence on pH and the molar ratio, they identified reaction products with co-ordination through O; O_2; O + N_{amine}; $N_{amine\,2}$; N_{amine}, N_{amide}; N_{amine}, $O_{peptide}$ + N_{amide}, O. Slow hydrolysis of the peptide bond was observed for the N_{amine}, $O_{peptide}$ species similarly as for other peptides in the presence of Pt^{II}[30,31] or Pd^{II}.[32]

Comparing our results with those for Gly and Gly-Gly and other common dipeptides, it is evident that the complexing properties of Pd^{II} and Pt^{II} for Gly-(*P*) and Gly-Gly-(*P*) are very similar to those of their carboxylic analogues.

Experimental

The compound Gly-(*P*) was prepared according to Soroka[33] and Gly-Gly-(*P*) according to our previous paper.[34]

Potentiometric titrations

The titration procedure and calculation of the protonation constants was described previously.[15] Stock solutions of Pt^{II} and Pd^{II} were made from $K_2[PtCl_4]$ and $K_2[PdCl_4]$ (p.a., Safina). Nitric acid was prepared by passing potassium nitrate through a Dowex 50W column in the H^+ form, because of traces of NO and NO_2 in the concentrated acid. The palladium and platinum contents in the solutions were determined gravimetrically after reduction to the metals with sodium formate.

The palladium(II) and platinum(II) systems were titrated by the 'out of cell' method using a PHM 84 pH-meter, ABU 80 automatic burette and a GK 2401 B combined electrode (Radiometer) in 60 tubes and thermostatted at 25 ± 0.1 °C and at an ionic strength of I (KNO_3) = 0.1 mol dm^{-3} in the region pH 1.7–11. However, only data from the region pH 2–9 were considered. The titration solutions were prepared by neutralisation of a solution containing nitric acid and the phosphonic acid by the appropriate volume of potassium hydroxide solution under an argon atmosphere. Then a solution of $K_2[PtCl_4]$ or $K_2[PdCl_4]$ was added, still under argon. Using this procedure we avoid formation of the hydroxo-complexes and subsequent reduction to the metals. The initial volume was 1 cm^3 and the concentration of the metal was 0.005 mol dm^{-3}. The metal : phosphonic acid molar ratio was 1:2 and 1:4 for both acids. Each titration was carried out at least twice. The total number of data points was over 100 for each ratio. The Pt^{II}–Gly-(*P*) system was also studied at a higher concentration. The phosphonic acid concentration was 0.3 mol dm^{-3} and that of KNO_3 was 0.3 mol dm^{-3}. An inert atmosphere during measurement was ensured by constant passage of argon saturated with the vapour of the solvent. The stability constants β_{pqrs} are the concentration constants defined as $[M_pA_qH_rCl_s]/[M]^p[A]^q[H]^r[Cl]^s$. They were refined by our program[35] which minimises the criterion of the generalised least-squares method. The program includes the calibration function $E = E_\circ - S(-\log [H^+]) + j_a[H^+] + j_b(K_w/[H^+])$ where the additive term $E_\circ$ contains the standard potentials of the electrodes used and the contributions of inert ions to the liquid-junction potential, S corresponds to the Nernstian slope, the value of which should be close to the theoretical value, and $j_a[H^+]$ and $j_b[OH^-]$ are the contributions of the H^+ and OH^- ions to the liquid-junction potential. It is clear that j_a and j_b cause deviations from a linear dependence between E and $-\log [H^+]$ only in strong acid and strong base. The procedure was tested by the 'glycine test'.[36]

NMR spectra

Proton NMR spectra for the titration were run with a Varian XL-200 instrument (200 MHz) at 25 °C with sodium 4,4-dimethyl-4-silapentanesulfonate as the internal standard. The standard was added to the solution just before measurement to avoid any influence on the equilibria of the systems. The ^{31}P-{^1H} NMR titration measurements were carried out using a Varian XL-200 instrument (81 MHz) and with 85% H_3PO_4 as the external standard. The concentration of metals was between 0.075 and 0.1 mol dm^{-3} in D_2O. The solutions were prepared by dissolving the dipeptide in D_2O, neutralising with KOD to the pD values estimated and then the appropriate amount of solid $K_2[PtCl_4]$ or $K_2[PdCl_4]$ was added. The final pD values (pD = pH + 0.40) were determined after attainment of equilibrium. The ^{195}Pt NMR spectrum of one sample was measured with a Varian 400 Inova instrument.

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