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pH-METRIC INVESTIGATION ON MIXED-LIGAND COMPLEXES OF Pd(II) WITH AMLODIPINE DRUG AS THE PRIMARY LIGAND AND PEPTIDES AS THE SECONDARY LIGAND

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ABSTRACT

A potentiometric titration technique has been used to determine the stability constants for various complexes of$[Pd(drug)(H_2O)_2]^{2+}$with peptides(L), e.g., glycylglycine, leucylalanine, glycylvaline, glycinamide and glutamine. The stepwise formation of the complexes has been established in the pH region studied. Stoichiometry and stability constants of the complexes were determined under physiologically similar conditions of37°C and 0.1 mol dm^{-3} of $NaNO_3$.

Keywords: Drug; Potentiometric studies; Solution equilibria; Stability constants; peptides

1. INTRODUCTION

Medicinal uses and applications of metals and metal complexes are of increasing clinical and commercial importance. The increasing numbers of research articles and major reviews, as well as dedicated volumes, testify to the growing importance of the discipline.[1–11]A list of clinically used chelating agents may be found in most pharmacopoeias,[12]while new chelating agents continue to be sought out.[12,13]The use of chelating agents in the treatment of Wilson's disease is a good example of how medical problems due to free metal ion (CuII) toxicity may be ameliorated by chelating agents.[14]Likewise, the extensive work on matrix metallo-proteinases represents a case study on the

design of small organic ligands as drugs to deactivate metalloenzymes. [15,16] Overexpression of these zinc-containing enzymes is associated with several diseases, including arthritis and cancer; thus, inhibition of the zinc active site is a reasonable drug development strategy. Indeed, enzymatic zinc is an attractive target because of the diversity of its structural and catalytic roles in enzymes.[17,18] Cisplatin (cis-$[PtCl_2(NH_3)_2]$ or cis-DDP) is perhaps the best known example of a small molecule metal-containing drug. Both Pd(II) and Pt(II) are soft Lewis acids and form stronger bonds with nitrogen or sulfur donors (soft bases) than oxygen donors (hard bases). In terms of complex formation and acid dissociation constants, Pt(II) and Pd(II) complexes behave very similarly, regardless of the fact that Pd(II) complexes are approximately 10^3-10^5 times faster than the corresponding Pt(II) complexes. This last property explains the lower anti-tumor activities of *cis*-$Pd(en)Cl_2$ and *cis*-$Pd(DACH)_2Cl_2$ when compared to the analogous Pt(II) complexes, as well as their high toxicities[19]. In general, the use of Pd(II) and its complexes in medicine has been limited. The only application is using 10^3Pd as a radioactive isotope in the treatment of rapidly growing high-grade prostate cancer.[20, 21] However, Pd(II) N, S chelates with inert ligands (e.g., sulfur or nitrogen) have been suggested by Das and Livingstone [22] to be more effective anti-tumor agents than those of other metals in that they possess the proper labilities to bring the metal to the target (DNA), followed by subsequent interactions. The Pd(II) complex of tetracycline drug as the ligand is practically as efficient as tetracycline in inhibiting the growth of two *E. coli* sensitive bacterial strains and 16 times more potent against *E. coli* HB101/pBR322, a bacterial strain resistant to tetracycline[23]. In light of the growing interest of ternary complexes, it is worthwhile to study the ternary complexes of a number of peptides and amlodipinedrug with Pd (II).As shown in Fig. 1, Amlodipine or2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester is a second generation 1,4-dihydropyridine derivative of the prototypical molecule nifedipine.[24]Amlodipine is used in the treatment of chronic stable angina and in

the management of mild-to-moderate essential hypertension. In the present study, equilibrium studies of the interaction between $[Pd(drug)(H_2O)_2]^{2+}$ and some peptidesare conducted using a potentiometric method under physiological-like conditions (0.1 mol $dm^{-3}$of $NaNO_3$ at 37°C). The concentration distribution relations of the various complex species will be evaluated.

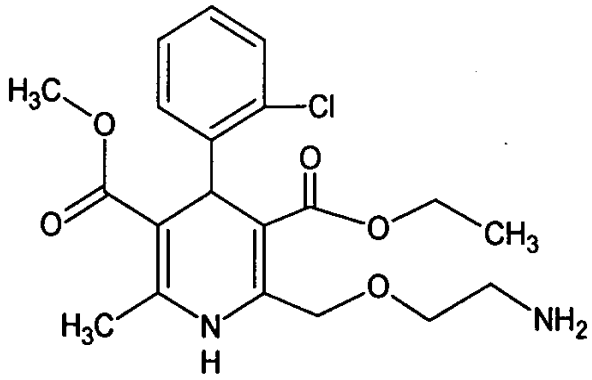

Fig. 1: Chemical structure of Amlodipine.

2. EXPERIMENTAL

2.1. Materials and Reagents

All chemicals used were of analytical grade. Amlodipine, $PdCl_2$ and peptides, i.e.,glycylglycine, leucylalanine, glycylvaline, glycinamide and glutamine, were provided by Sigma-Aldrich (Germany). All of these chemicals were used as received without any further purification, and their purities ranged from 99 to 99.9%. Carbonate-free sodium hydroxide, i.e., the titrant solution, was standardized potentiometrically with potassium hydrogen phthalate (Merck Chem. Co.). All solutions were prepared with bi-distilled water.

2.2. Potentiometric Procedure and Measurements

For the determination of the acid dissociation constants of glycylglycine, leucylalanine, glycylvalineand glutamine ligands, aqueous solutions (1.25×10^{-3} mol dm^{-3}) of the ligands were titrated with 0.1 mol dm^{-3}NaOH at 37.0 ºC under an ionic strength of a 0.1 moldm^{-3} solution of $NaNO_3$.

For the equilibrium studies, $[Pd(drug)Cl_2]$ was prepared as described before.[25] The diaqua complex $[Pd(drug)(H_2O)_2]^{2+}$was prepared in solution by stirring the chloro complex with two equivalents of $AgNO_3$overnight (under careful protection from light). The precipitated

AgCl was removed by filtration, and the filtrate was increased up to the desired volume in a standard volumetric flask. The acid dissociation constants of the coordinated water molecules in [Pd (drug)$(H_2O)_2)]^{2+}$ were determined by titrating a 1.25×10^{-3}mol dm^{-3} solution of the complex. The formation constants of the complexes were determined by titrating solution mixtures of $[Pd(drug)(H_2O)_2)]^{2+}$(1.25×10^{-3}mol dm^{-3}) and the ligand in a concentration ratio of 1:1. The titration solution mixtures had a volume of 40 cm^3.The ionic strength was adjusted to 0.1 mol dm^{-3} by using $NaNO_3$. A 0.10 mol dm^{-3} NaOH solution was used as the titrant. The equilibrium constants evaluated from the titration data (summarized in Table 1) are defined by Eqs. (1) and (2), where M, L and H stand for the $[Pd(drug)(H_2O)_2)]^{2+}$ ion, ligand and proton, respectively.

$$pM+qL+rH \rightleftharpoons [MpLqHr\ ] \quad (1)$$

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

Potentiometric titrations were performed at 25°C±0.1°C in a double-walled glass vessel using a Griffin pH J-300-010 G Digital pH meter. The temperature was controlled by circulating water through the jacket from a constant temperature bath. The electrode system was calibrated in terms of the hydrogen-ion concentrations instead of the activities.

2.3. CALCULATIONS

The calculations were performed using approximately 100 data points in each titration using the HYPERQUAD program.[26] The stoichiometry and stability constants of the complexes formed were determined by trying various possible composition models. The results obtained are shown in Table 1. The concentration distribution diagrams were obtained using the HYSS [27]program under the experimental conditions used.

3. RESULTS AND DISCUSSION

The acid dissociation constants of the ligands were determined under the experimental conditions of 37°C and a constant ionic strength of 0.1 mol dm^{-3} $NaNO_3$, which were also used to determine the stability constants of the Pd(II) complexes.

Acid–base equilibria of [Pd (drug)$(H_2O)_2]^{+2}$

The complex,$[Pd(drug)(H_2O)_2]^{+2}$ ion, may undergo hydrolysis.(28) Its acid-base chemistry was characterized by fitting the potentiometric data to various acid-base models. The best fit model was found to

be consistent with the species of the compositions 10-1, 10-2 and 20-2, as given in Reactions (3) to (5).

$$[Pd(drug)(H_2O)_2]^{2+} \rightleftharpoons [Pd(drug)(H_2O)(OH)]^{+} + H^{+} \quad (3)$$
100 **10-1**

$$[Pd(drug)(H_2O)(OH)]^{+} \rightleftharpoons [Pd(drug)(OH)_2] + H^{+} \quad (4)$$
10-1 **10-2**

$$2\,[Pd(drug)(H_2O)(OH)]^{+} \rightleftharpoons [Pd(drug)(OH)_2pd(drug)]^{2+} + 2H^{+} \quad (5)$$
10-1 **20-2**

The pK_{a1} and pK_{a2} values were found to be 4.48 and 7.67, respectively. The equilibrium constant for the dimerization reaction (5) can be calculated with Eq. (6), which gives 2.09.

$$log\ K_{dimer} = log\ \beta_{20-2} - 2\ log\ \beta_{10-1} \quad (6)$$

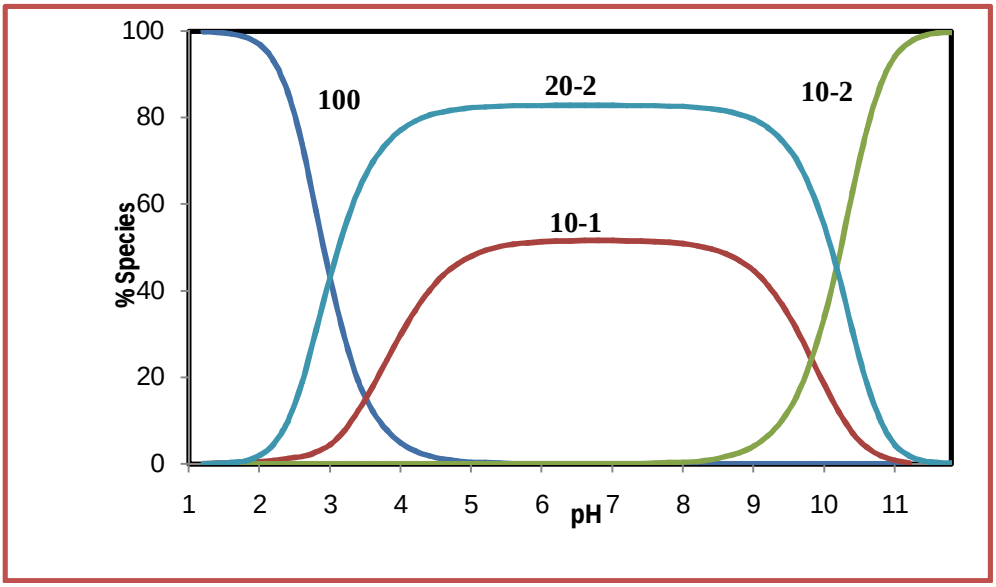

Fig. 2.Distribution of various species as a function of pH in the hydrolysis of thePd(drug)$(H_2O)_2$system.

The species distribution diagram for $[Pd(drug)(H_2O)_2]^{2+}$ and its hydrolyzed species is shown in Fig 2. The concentrations of the monohydroxo (10-1) and dimeric (20-2) species increased with the increase of pH, attaining a maximum of 51.63 and 82.85% inthe pH range of ca. 6 to 8, respectively, i.e., they were the main species present in the solution in the physiological pH range. A further increase in the pH was accompanied by a decrease in the concentration of the monohydroxo species and an increase in the concentration of the dihydroxo species, which is the main species above a pH of *ca.* 10.

Ternary Pd(II) complexes

A representative potentiometric equilibrium titration curve for the

Pd(DAP)-glycylglycine system was significantly lower than the glycylglycine titration curve(Fig. 3). This corresponded to the formation of a complex species via the release of a hydrogen ion.

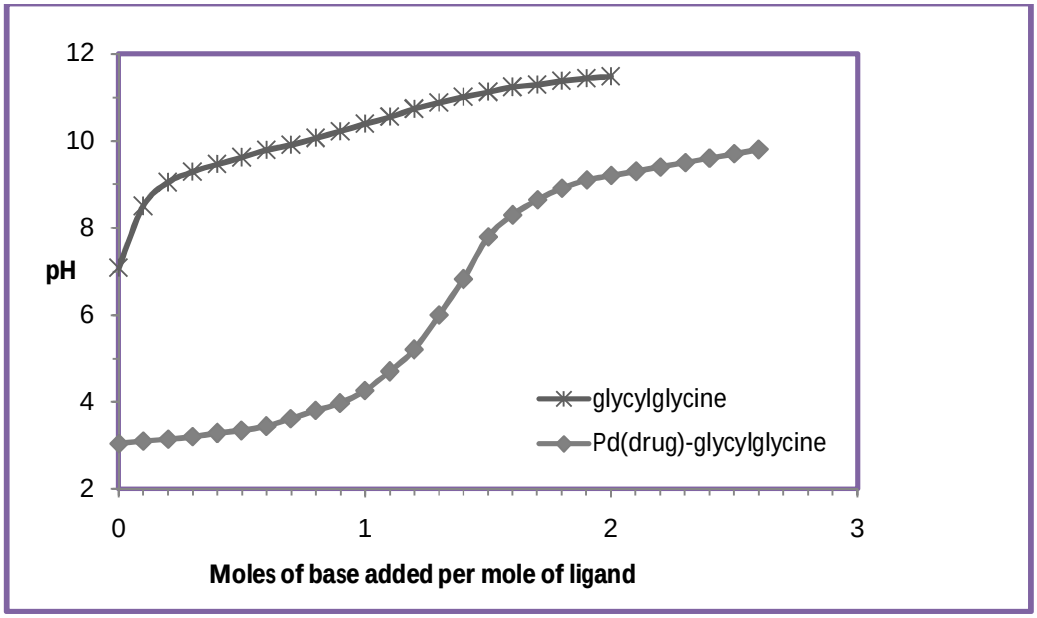

Fig. 3. Potentiometric titration curves of the Pd(drug)-glycylglycine system.

The potentiometric data of the Pd(drug) peptide system were fitted by various models. The most acceptable model was found to be consistent with the formation of complexes with the stoichiometric coefficients of110and 11-1.In the 110case, the peptide was bound through the amino and carbonyl oxygen groups. The complexes were formed by the coordination of the amino and carbonyl groups. Upon de-protonation of the amide group, the coordination sites switched from carbonyl oxygen to amide nitrogen. Such changes in the coordination centers have been well documented.[29]

The peptides, i.e., glycylglycine, leucylalanine and glycylvaline, may coordinate through the terminal amino and carboxyl groups with the amide group in the side chain. The ligands would then behave like a simple amino acid. Alternatively, they can also coordinate through the terminal amino group and the carbonyl oxygen of the amide group. A third possibility, involving the carboxyl and the amide groups, can be safely ruled out due to the large affinity of palladium to the nitrogen

donor centers. There is no conclusive evidence so far to exclude either one of these two aforementioned possibilities. From the fact that $log\beta_{110}$ for the peptide complexes compared favorably with that of the α-amino acids and if the differences in their basicities are considered, it can be suggested that the peptides probably coordinated as simple amino acids. The glutamine complex was more stable than the glycinamide complex and can be explained by the fact that glutaminate carried a negative charge, whereas glycinamide was neutral. The electrostatic interaction between glutaminate and the two-fold positively charged metal complex would result in a lowering of the free energy of formation.

The pK^H values of the amide groups incorporated in the Pd(II) complexes $(log\beta_{110} - log_{10}\beta_{11-1})$ were in the range of 4.67 to 10.45 (*see* Table 1). It is worth to note that the pK^H for the glutamine complex was relatively much higher than the others. This was due to the formation of a seven-membered chelatering, which would be more strained and less favored. Therefore, under physiological conditions (pH~7.4), glutamine would coordinate in its protonated form.

The relative magnitudes of $pK^H$of the Pd(II) complexes with peptides have interesting biological applications. Under normal physiological conditions (pHofca.7.4), $[Pd(drug)(H_2O)_2]^{2+}$can catalyze the deprotonation of the amide group. The catalytic action would be very specific under physiological conditions. The slight difference in the side chain of the peptides will produce dramatic differences in their behaviors towards the palladium species.

The concentration distribution diagram for the [Pd(drug)-glycylglycine] (110) system is given in Fig. 4. [Pd(drug)L] started to form at a pH of 1.4, and its concentration increased with increasing pH, reaching a maximum of 93.7% at a pH of ca. 6.8. A further increase of pH was accompanied by a decrease in the [Pd(drug)L] concentration and an increase of [Pd(drug)LH-1] (11-1)], reaching a maximum of 96% ata pH of10.8.

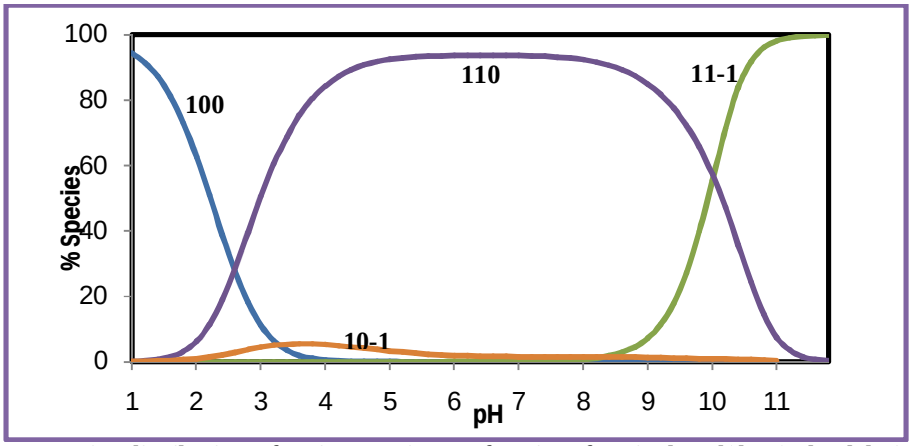

Fig. 4: Concentration distributions of various species as a function of pH in the Pd(drug)-glycylglycine system.

Table (1): Formation constants of Pd(drug) complexes with peptidesat 37°C and 0.1 mol dm^{-3} $NaNO_3$ionic strength

System	*p*	q	r[a]	*logβ*[b]	pK^H
Pd(drug)-OH	1	0	-1	-4.48(0.01)	
	1	0	-2	-12.15(0.02)	
	2	0	-2	-6.87(0.02)	
Glycylglycine	0	1	1	7.97(0.00)	
	1	1	0	9.09(0.01)	
	1	1	-1	3.13(0.02)	5.96
leucylalanine	0	1	1	8.13(0.02)	
	1	1	0	10.34(0.01)	
	1	1	-1	3.43(0.03)	6.91
Glycylvaline	0	1	1	8.24(0.01)	
	1	1	0	10.23(0.02)	
	1	1	-1	4.11(0.01)	6.12
Glutamine	0	1	1	8.98(0.00)	
	1	1	0	12.77(0.02)	
	1	1	-1	2.32(0.01)	10.45
Glycinamide	0	1	1	7.88(0.00)	
	1	1	0	10.52(0.02)	
	1	1	-1	5.89(0.01)	4.67

[a]*l, p* and *q* are the stoichiometric coefficients corresponding to Pd(drug), L and H^+,respectively. [b]Standard deviations are given in parentheses.

CONCLUSION

The potentiometric data for the system consisting of [Pd(drug)] and peptides showed the formation of complexes with stoichiometric coefficients of 110and 11-1. The pK^H value, i.e.,$(log\beta_{110} - log_{10}\beta_{11-1})$, for the glutamine complex was higher than that of the other peptide complexes. This was due to the formation of a seven-membered chelatering, which would be more strained and less favored. Therefore, under physiological conditions (pH~7.4), glutamine would coordinate in its protonated form. The concentration distribution curves of the various complex species existing in solution were evaluated.

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