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Correlation between kinetic and thermodynamic complex-formation constants for the interaction of bis(amine)palladium(II) with inosine, inosine 5′-monophosphate and guanosine 5′-monophosphate

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The complex-formation equilibria of [Pd(amine)Cl_2] (amine = *N*,*N*,*N*′,*N*′-tetramethyl- or *N*,*N*,*N*′,*N*′-tetraethyl-ethane-1,2-diamine) with inosine, inosine 5′-monophosphate, guanosine and guanosine 5′-monophosphate were investigated at different temperatures using a potentiometric technique. The stepwise formation constants of the complexes formed in solution were calculated using the non-linear least-squares program MINIQUAD 75. The mode of binding of the DNA unit to the palladium(II) complexes is discussed. Comparison of the potentiometric results with corresponding data obtained from kinetic measurements indicates that atom N^7 constitutes the binding site in acidic media and N^1 in basic media. The concentration distribution of the various complex species was evaluated as a function of pH.

Work in our laboratories in recent years [1–7] has focused on the mechanistic behaviour of model *cis*-bis(amine)palladium(II) complexes in their interaction with nucleosides and 5′-nucleotides in reference to the antitumour behaviour of related platinum(II) complexes. Our kinetic studies have revealed a richness of mechanistic versatility for ligand displacement and complex-formation reactions in these systems, which is controlled by steric hindrance on the palladium(II) complex and the nucleophilicity of the DNA constituent. With all the kinetic information now available it is possible to extract thermodynamic data on the complex-formation equilibria and to compare these with data measured directly. Such correlations in the past have shown to be in good agreement in the case of complex formation with small and simple nucleophiles.[8–10] However, in the case of nucleosides and 5′-nucleotides there are various co-ordination sites available that could strongly affect both the kinetic and overall thermodynamic data for such complex-formation reactions.

The comparison of kinetic and thermodynamic equilibrium data is of fundamental importance for an improved understanding of the antitumour activity of *cis*-bis(amine)-platinum(II) complexes.[11] Although rate constants in the case of these complexes are usually four to five orders of magnitude slower than for corresponding palladium(II) complexes, the overall equilibrium constants are almost identical since both reactions constituting the equilibrium are affected to the same degree.[9,11]

Inosine, inosine 5′-monophosphate, guanosine and guanosine 5′-monophosphate can co-ordinate to metal ions atoms *via* N^1 and N^7. Our earlier kinetic studies were performed in weakly acidic media under which conditions N^7 is the preferred binding site. In the present study we have studied the acid–base equilibria and the complex-formation constants of *cis*-[Pd(tmen)Cl_2] and *cis*-[Pd(teen)Cl_2] (tmen = *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine, teen = *N*,*N*,*N*′,*N*′-tetraethylethane-1,2-diamine) with inosine, inosine 5′-monophosphate, guanosine and guanosine 5′-monophosphate using potentiometric techniques. The palladium(II) complexes were mainly used in the dichloro form in order to approach to some degree the physiological conditions: the chloride-ion concentration is 0.1 mol dm^{-3} in blood and 4 mmol dm^{-3} in the cell. In the case of the tmen complex, experiments were also performed with the diaqua form of the complex. A correlation with the data obtained from the kinetic measurements reveals large discrepancies that can be assigned to the pH-dependent N^7 *versus* N^1 co-ordination of the DNA constituents. A systematic variation of pH allowed an analysis of the role of N^1 co-ordination. The results enable a discussion of the basicity of co-ordinated nucleosides/nucleotides and a comparison with available literature data.[12] The intrinsic difference in the kinetic and thermodynamic approach to determine overall equilibrium constants is highlighted and sets a guideline for future work in this area.

Experimental

Materials and reagents

The [Pd(tmen)Cl_2] and [Pd(teen)Cl_2] complexes were prepared and characterized as described before.[9] The DNA constituents (Nuc) inosine, inosine 5′-monophosphate, guanosine and guanosine 5′-monophosphate were obtained from Sigma and used without further purification.

Procedure and measurements

Potentiometric measurements were performed on a Metrohm 686 Titroprocessor equipped with a 665 Dosimat (Switzerland). The titroprocessor and electrode were calibrated with standard buffer solutions prepared according to NBS specifications.[13] Spectral measurements and stopped-flow kinetic studies were performed on the instrumentation outlined before.[5] All kinetic measurements were performed under pseudo-first-order conditions, *i.e.* an excess of the nucleoside or nucleotide was employed. The OLIS KINFIT set of programs[14] was employed for analysis of the absorbance *vs.* time traces.

The following mixtures (A)–(C) were prepared and titrated with 0.091 mol dm^{-3} NaOH: (A) 10 cm^3 of 0.01 mol dm^{-3} Nuc + 30 cm^3 of 0.33 mol dm^{-3} $NaClO_4$; (B) 30 cm^3 of 0.001 mol dm^{-3} palladium(II) complex + 10 cm^3 of 0.40 mol dm^{-3} $NaClO_4$; (C) 10 cm^3 of 0.01 mol dm^{-3} Nuc + 20 cm^3 of 0.001 mol dm^{-3} palladium(II) complex + 10 cm^3 of 0.40 mol dm^{-3} $NaClO_4$. All titrations were carried out in a purified nitrogen atmosphere in a special vessel described previously.[15] The acid-dissociation constants of the Nuc were determined by titrating mixture (A). The acid–base equilibria of the palladium(II) complexes were characterized by titrating mixture

(B). The stability constants of the palladium–Nuc complexes were determined by titrating mixture (C).

The equilibrium constants were evaluated from titration data. They are defined by equations (1) and (2) where M′, L

$$pM' + qL + rH \rightleftharpoons M'_pL_qH_r \quad (1)$$

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

and H represent Pd(tmen) or Pd(teen), Nuc and proton, respectively. The calculations were performed using the computer program MINIQUAD 75[5] on an IBM 486 computer. The stoichiometries and stability constants of the complexes formed were determined by trying various possible composition models for the system studied. The calculations were restricted to data obtained in a pH range where no precipitation occurred. The model selected was that which gave the best statistical fit and which was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals, as described elsewhere.[16] Table 1 lists the equilibrium constants together with their standard deviations and the sum of the square of residuals as obtained from the program MINIQUAD 75. The concentration distribution diagrams were obtained with the program SPECIES[17] under the experimental conditions used.

Results and Discussion

Inosine, inosine 5′-monophosphate and guanosine 5′-monophosphate bear a dissociable proton at N^1. Their titration curves showed a steep inflection at 1:1 hydroxide-to-Nuc stoichiometry. The pK_a values obtained were in good agreement with literature data.[12,18]

The $[Pd(tmen)Cl_2]$ and $[Pd(teen)Cl_2]$ complexes may undergo spontaneous hydrolysis. Their acid–base chemistry was characterized by fitting the potentiometric data [mixture (B)] by various acid–base models. The fitted model was found to be consistent with the $Pd(tmen)(OH)_n$ and $Pd(teen)(OH)_n$ species, where $n = 1$ or 2. The concentration distribution diagram of the $Pd(teen)(OH)_n$ system is shown in Fig. 1. The concentration of the monohydroxo species increases with increasing pH, attaining a maximum of 99% at pH 7.0. Further increase in pH is accompanied by a decrease in this species and an increase in the dihydroxo species concentration. This reveals that in the physiological pH range, *i.e.* at pH 6–7, the monohydroxo complex is predominant and can interact with the DNA subunits. At high pH the inert dihydroxo complex will be the major species and consequently the ability of DNA to bind the Pd(amine) complexes will decrease significantly.

The potentiometric equilibrium titration curve of the Pd(amine)–Nu system [mixture (C)] is significantly lower than the Nuc titration curve (see Fig. 2). This corresponds to the formation of a complex species through release of a hydrogen ion, most propably from N^1H. The combined results for all Nucs investigated show the formation of 1:1 and 1:2 (Pd:Nuc) complex species. There was no evidence for the formation of dimeric complexes from the refinement of the titration data. Estimation of the concentration distribution of the various complex species in solution provides a useful picture of metal-ion binding toward DNA. To illustrate the main features observed in the species distribution plots in these systems the speciation diagram obtained for the Pd(teen)–IMP system is shown in Fig. 3. In all the species distributions the concentration of the complex increases with increasing pH, thus favouring complex formation with DNA in the physiological pH range. Under the selected experimental conditions, the magnitude of the stability constants controls the concentration distribution of the different species. The stability constant for the formation of the 1:1 complex is significantly larger for inosine than for the monophosphates, with the result that the inosine complex starts to form at pH 5, whereas the monophosphate complexes only start to form at pH 7. This can partially be assigned to the significantly different size and

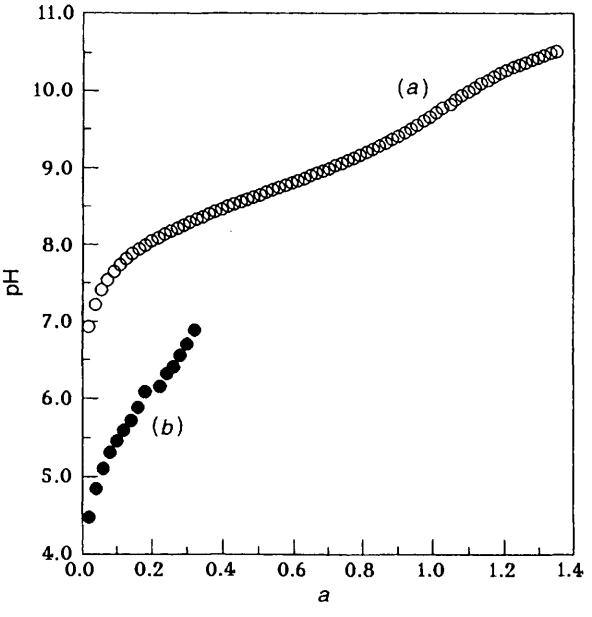


Fig. 2 Typical potentiometric titration curves for the Pd(teen)–inosine system at 25.0 °C and 0.1 mol dm^{-3} ionic strength: (*a*) free inosine, (*b*) inosine complex; '*a*' represents the number of moles of base added per mole of inosine. For experimental conditions see text

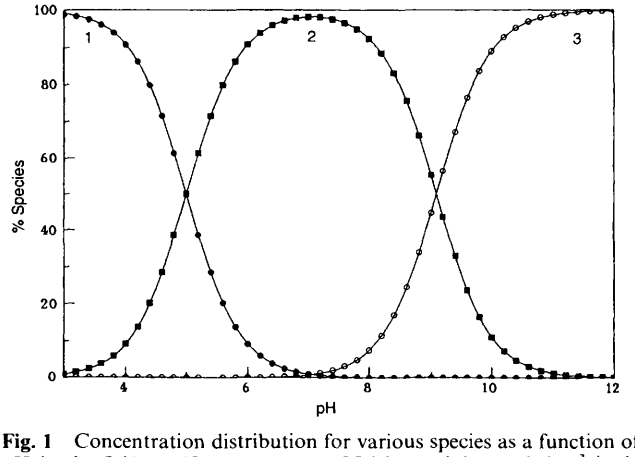


Fig. 1 Concentration distribution for various species as a function of pH in the $Pd(teen)(OH)_n$ system at 25.0 °C and 0.1 mol dm^{-3} ionic strength. Species: 1, M′; 2, M′(OH); 3, $M'(OH)_2$, where M′ = Pd(teen)

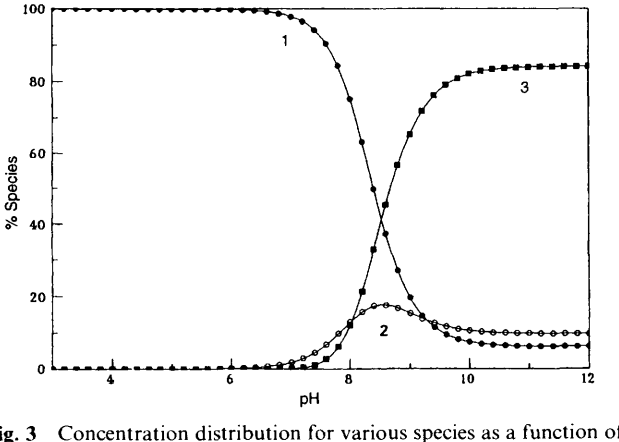


Fig. 3 Concentration distribution for various species as a function of pH in the Pd(teen)–IMP system at 25.0 °C and 0.1 mol dm^{-3} ionic strength. Species: 1, M′; 2, M′(IMP); 3, $M'(IMP)_2$, where M′ = Pd(teen)

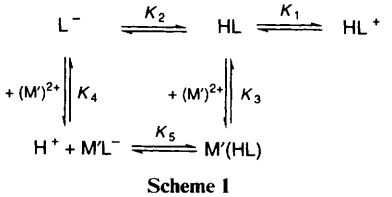
Table 1 Equilibrium constants for inosine, inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP) and *cis*-bis(amine)palladium(II) complexes

System	*l*	*p*	*q*[a]	log β[b]	*S*[c]	*T*/°C
Inosine–H^+	0	1	1	8.77		15.0
	0	1	1	8.67		20.0
	0	1	1	8.55		25.0
	0	1	1	8.44		30.5
	0	1	1	8.37		35.0
IMP–H^+	0	1	1	9.33		15.0
	0	1	1	9.17		20.0
	0	1	1	9.09		25.0
	0	1	1	8.95		30.0
	0	1	1	8.89		35.0
GMP–H^+	0	1	1	9.81		15.0
	0	1	1	9.58		20.0
	0	1	1	9.43		25.0
	0	1	1	9.23		30.0
	0	1	1	9.16		35.0
Pd(tmen)–OH	1	0	−1	−5.96 (0.02)	2.6×10^{-8}	15.5
	1	0	−2	−15.75 (0.03)		
	1	0	−1	−5.89 (0.02)	1.9×10^{-8}	20.0
	1	0	−2	−15.48 (0.03)		
	1	0	−1	−5.71 (0.02)	2.9×10^{-8}	25.7
	1	0	−2	−15.03 (0.04)		
	1	0	−1	−5.66 (0.02)	3.2×10^{-8}	30.0
	1	0	−2	−14.82 (0.04)		
	1	0	−1	−5.57 (0.02)	3.3×10^{-8}	35.2
	1	0	−2	−14.69 (0.04)		
Pd(teen)–OH^-	1	0	−1	−5.25 (0.05)	3.5×10^{-7}	15.0
	1	0	−2	−14.61 (0.15)		
	1	0	−1	−5.17 (0.04)	1.8×10^{-7}	20.0
	1	0	−2	−14.49 (0.10)		
	1	0	−1	−5.00 (0.04)	2.6×10^{-7}	25.0
	1	0	−2	−14.09 (0.10)		
	1	0	−1	−4.94 (0.03)	1.1×10^{-7}	30.0
	1	0	−2	−14.25 (0.07)		
	1	0	−1	−4.87 (0.03)	4.0×10^{-7}	35.0
	1	0	−2	−14.12 (0.08)		
Pd(tmen)–Ino	1	1	0	5.91 (0.11)	1.5×10^{-8}	15.5
	1	2	0	9.57 (0.15)		
	1	1	0	5.81 (0.09)	8.8×10^{-9}	20.0
	1	2	0	9.11 (0.14)		
	1	1	0	6.04 (0.07)	6.1×10^{-9}	25.7
	1	2	0	9.56 (0.10)		
	1	1	0	5.99 (0.08)	7.9×10^{-9}	30.0
	1	2	0	9.30 (0.13)		
	1	1	0	6.06 (0.04)	2.4×10^{-9}	35.2
	1	2	0	9.30 (0.07)		
Pd(tmen)–IMP	1	1	0	4.93 (0.08)	5.1×10^{-8}	15.0
	1	2	0	8.79 (0.10)		
	1	1	0	4.84 (0.07)	3.5×10^{-8}	20.0
	1	2	0	8.91 (0.07)		
	1	1	0	4.43 (0.13)	2.8×10^{-8}	25.0
	1	2	0	9.20 (0.04)		
	1	1	0	4.19 (0.13)	2.4×10^{-8}	30.0
	1	2	0	8.89 (0.04)		
	1	1	0	3.95 (0.18)	2.3×10^{-8}	35.0
	1	2	0	8.76 (0.03)		
Pd(tmen)–GMP	1	1	0	4.19 (0.02)	1.4×10^{-9}	15.0
	1	2	0	8.31 (0.01)		
	1	1	0	4.12 (0.02)	1.4×10^{-9}	20.0
	1	2	0	8.21 (0.01)		
	1	1	0	4.14 (0.03)	2.9×10^{-9}	25.0
	1	2	0	8.03 (0.02)		
	1	1	0	4.19 (0.07)	1.6×10^{-8}	30.0
	1	2	0	8.22 (0.04)		
	1	1	0	3.98 (0.09)	2.0×10^{-8}	35.0
	1	2	0	8.09 (0.04)		
Pd(teen)–Ino	1	1	0	5.64 (0.03)	1.3×10^{-8}	15.0
	1	2	0	10.23 (0.03)		
	1	1	0	5.83 (0.03)	1.1×10^{-8}	20.0
	1	2	0	10.74 (0.02)		
	1	1	0	5.78 (0.03)	2.8×10^{-9}	25.0
	1	2	0	10.48 (0.03)		
	1	1	0	5.93 (0.03)	1.1×10^{-8}	30.0
	1	2	0	10.69 (0.03)		
	1	1	0	6.04 (0.03)	1.1×10^{-8}	35.0
	1	2	0	10.77 (0.03)		
Pd(teen)–IMP	1	1	0	4.62 (0.13)	2.7×10^{-8}	15.0
	1	2	0	10.17 (0.02)		
	1	1	0	4.54 (0.23)	1.6×10^{-8}	20.0
	1	2	0	10.08 (0.04)		
	1	1	0	4.39 (0.22)	1.6×10^{-8}	25.0
	1	2	0	9.73 (0.04)		
	1	1	0	4.39 (0.25)	3.2×10^{-8}	30.0
	1	2	0	9.52 (0.02)		
	1	1	0	3.61 (0.45)	2.3×10^{-8}	35.0
	1	2	0	9.54 (0.02)		
Pd(teen)–GMP	1	1	0	4.72 (0.05)	2.5×10^{-8}	15.0
	1	2	0	8.45 (0.04)		
	1	1	0	4.36 (0.06)	8.4×10^{-9}	20.0
	1	2	0	7.89 (0.05)		
	1	1	0	4.00 (0.04)	3.3×10^{-9}	25.0
	1	2	0	7.14 (0.04)		
	1	1	0	3.99 (0.10)	8.7×10^{-9}	30.0
	1	2	0	7.22 (0.03)		
	1	1	0	3.79 (0.03)	8.4×10^{-9}	35.0
	1	2	0	7.16 (0.02)		

[a] *l*, *p* and *q* are the stoichiometric coefficients corresponding to Pd(amine), Nuc and H^+, respectively. [b] Standard deviations are given in parentheses. [c] Sum of squares of residuals.

charge of the entering nucleophiles which will affect the overall equilibrium constants. The titration data were also fitted with models in which complex formation of the hydroxo complexes was taken into consideration. The data fits, however, were very poor and indicated that such species are weak complex-formation partners, which is consistent with the known inertness of such species from kinetic measurements.[1-7]

There is a dichotomy concerning the binding of metal ions to the nucleic base portion of purine nucleosides and nucleotides.[12] Both N^1 in the six-membered ring and N^7 in the imidazole ring of inosine, inosine 5′-monophosphate and guanosine 5′-monophosphate serve as donors to metal ions in solution.[19] Crystal structure determinations[20] have given an exaggerated picture of the extent of metal-ion binding to N^7 in purine nucleotides and nucleosides, due to the fact that crystals were collected from acidic solutions which protonate N^1 and so favour metal-ion binding to N^7. In order to avoid precipitation of metal-ion hydroxides, solutions are often prepared in acidic media.



Scheme 1

In the potentiometric titration of the complexes described in this investigation the pH was varied over a range from 4.5 to 8 for inosine and 7 to 9 for the monophosphates. It is, therefore, assumed that N^1 plays a role during complex formation. This is in agreement with Martin's conclusion,[21] that starting from pH 5–6 a migration of $Pt(dien)^{2+}$ (dien = diethylenetriamine) occurs from N^7 to N^1. The mechanism in Scheme 1 provides the

main pathway for metal-ion binding to N^7 and the subsequent transfer to N^1, where L^- and M′ represent the deprotonated form of for instance inosine and the Pd(amine) complex, respectively; K_1 and K_2 are acid-dissociation constants for N^7 and N^1, respectively; K_3 and K_4 are the stability constants assuming that N^7 and N^1 are the binding sites, respectively and K_5 is the acid-dissociation constant of the protonated complex formed through binding at N^7.

The stability constants summarized in Table 1 correspond to those for K_4 (*i.e.* N^1 binding site) for the formation of the 1:1 and 1:2 complexes of Pd(amine) with inosine (Ino), inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP). The stability constants for the more predominant 1:1 complexes follow the sequence Ino > IMP ≈ GMP, which is most probably related to the size of the ligand. Furthermore, the data in Table 1 demonstrate that the tmen derivatives of the palladium(II) complexes are more stable than the corresponding teen derivatives. This can be accounted for in terms of steric crowding between the ethyl groups and the entering nucleophile. Comparison of these formation constants with those extrapolated from the kinetic data referred to before indicates that they are one to two orders of magnitude larger. For instance, the formation constant for the 1:1 complex between Pd(teen) and Ino as obtained from the kinetic data is 180 dm^3 mol^{-1}, and those for the formation of the 1:2 complex from the 1:1 complexes between Pd(tmen) and Pd(teen) and IMP are 997 and 1166 dm^3 mol^{-1}, respectively.[5] This apparent discrepancy must be due to the fact that the kinetic measurements were performed in weakly acidic media, where co-ordination predominantly occurs *via* N^7, compared to N^1 under the more basic conditions employed for the potentiometric work.

In order to resolve this apparent discrepancy the Pd(tmen)–guanosine system was titrated as a typical example in acidic media [using equimolar solutions of guanosine, Pd(tmen) and $HClO_4$, all 0.25 mmol dm^{-3}, in 0.1 mol dm^{-3} $NaClO_4$]. The guanosine system has more favourable pK_a values for the potentiometric analysis than the corresponding inosine system, although their chemical behaviours are very similar. Under such conditions N^1 is protonated and N^7 is the only available binding site. The pK_a values of guanosine are 2.47 and 8.84 for N^7 and N^1, respectively, which are in close agreement with available literature data.[12,18] The stability constant under these conditions was found to be log K_3 = 3.27 ± 0.08. A detailed kinetic and mechanistic study of the complex-formation reaction of Pd(amine) with inosine, inosine 5′-monophosphate and guanosine 5′-monophosphate was performed in weakly acidic solution, where N^7 is the binding site. The log K_3 value obtained from the kinetic studies for the Pd(teen)–inosine system is 2.25.[2] This is in fair agreement with that obtained from the potentiometric study, especially when the difference in ligand basicity and the nature of the alkyl substituent are taken into account. Thus the pH range in which the potentiometric titrations are performed controls the nature of the complex species produced. In addition, co-ordination to N^7 is expected to lower the pK_a value for N^1,[12] which is represented by K_5 in the present study; K_5 can be calculated from the relationship $K_5 = K_4/K_2K_3$. By taking K_4 and K_2 from Table 1 and K_3 from kinetic data,[2] pK_5 has a value of 5.0 for the Pd(teen)–inosine system, which indicates that co-ordination to N^7 lowers the pK_a of N^1 in inosine by more than three units. A lowering of up to two units was reported for the co-ordination of IMP and GMP to a series of divalent alkaline earth and transition-metal elements.[12]

The sequence of binding the palladium(II) complex to the N^7 and N^1 donor sites of guanosine was investigated in more detail by analysing the titration data in a higher pH range where both N^7 and N^1 may participate in complex formation. Under the condition of equimolar solutions (see above), the data were fitted by assuming formation of the 1:1 complex (log β_{110} = 8.01 ± 0.05) and its protonated form (log β_{111} = 11.85 ± 0.06). In the protonated complex the additional proton is most likely attached to N^1 and guanine binds to Pd^{II} through N^7. In the deprotonated complex, formed at higher pH, the binding site is N^1. Under these conditions the 1:2 complex was not detected since it is present in too low a concentration. The speciation for the Pd(tmen)–guanosine system under these conditions is given in Fig. 4. The concentration of the N^7-bound complex increases on increasing the pH and reaches a maximum of 14.5% at pH 3.3. On increasing the pH further the concentration of this complex decreases and that of the N^1-bound complex increases to reach a maximum at pH 8.

When [Pd(amine)Cl_2] (amine = tmen or teen) is dissolved in slightly acidic aqueous solution (pH 4.5) it undergoes spontaneous solvolysis to produce [Pd(amine)Cl(H_2O)]$^+$ and [Pd(amine)(H_2O)$_2$]$^{2+}$, where the equilibrium distribution will depend on the chloride-ion concentration in solution. The overall reactions are given in (3) and (4), for which the

$$[Pd(amine)Cl_2] + H_2O \overset{K_6}{\rightleftharpoons} [Pd(amine)Cl(H_2O)]^+ + Cl^- \quad (3)$$

$$[Pd(amine)Cl(H_2O)]^+ + H_2O \overset{K_7}{\rightleftharpoons} [Pd(amine)(H_2O)_2]^{2+} + Cl^- \quad (4)$$

equilibrium constants have the values K_6 = (7.7 ± 0.8) × 10^{-3}, (9.5 ± 1.3) × 10^{-3}, (1.9 ± 0.3) × 10^{-2} and K_7 = (2.5 ± 0.1) × 10^{-4}, (3.1 ± 0.5) × 10^{-4}, (3.5 ± 0.2) × 10^{-4} mol dm^{-3} for amine = en, tmen and teen, respectively, at 25 °C and 0.10 mol dm^{-3} ionic strength.[10] A realistic extrapolation of the present study to biologically relevant conditions will require a study of the effect of [Cl^-] on the stability constants of the complexes. The results in Table 2 show that the stability constant of the 1:1 complex in the Pd(tmen)–inosine system tends to decrease on increasing [Cl^-]. This is accounted for on the basis that the concentration of the active species, the mono- and the di-aqua complexes, decreases on increasing [Cl^-], and this in turn will affect the stability of the complexes formed.

In order to study the influence of the chloride ions present in solution the complex-formation equilibria with inosine, inosine 5′-monophosphate and guanosine were also studied using the diaqua complex of $Pd(tmen)^{2+}$, which was prepared in solution from the dichloro complex by addition of $AgClO_4$.[1] Under the normal titration conditions ([Pd^{II}]:[Nuc] = 1:5) the data were fitted with the formation of 1:1 and 1:2 complexes. The

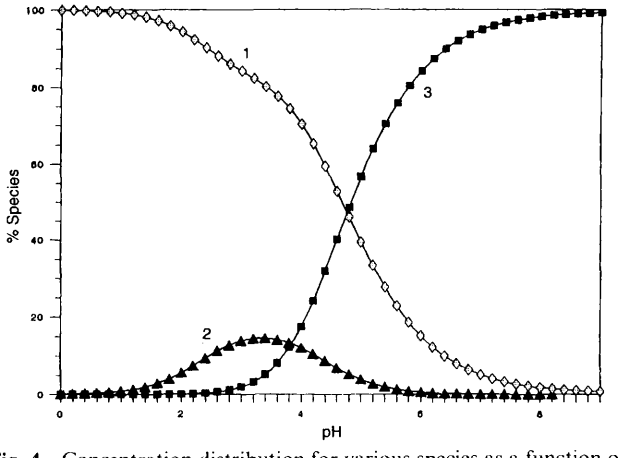


Fig. 4 Concentration distribution for various species as a function of pH in the Pd(tmen)–guanosine system at 25.0 °C and 0.1 mol dm^{-3} ionic strength. Species: 1, M′; 2, M′(Guo-N^7); 3, M′(Guo-N^1), where M′ = Pd(tmen)

Table 2 Equilibrium constants for Pd(teen)–inosine complexes as a function of chloride-ion concentration at constant ionic strength (0.2 mol dm $^{-3}$)

System	l	p	q	log β	S	[Cl $^-$]/mol dm $^{-3}$
Inosine	0	1	1	8.65		
Pd(tmen)–Ino	1	1	0	6.08 (0.02)	1.8×10^{-9}	0.05
	1	2	0	10.28 (0.03)		
	1	1	0	6.13 (0.04)	8.4×10^{-9}	0.10
	1	2	0	9.87 (0.06)		
	1	1	0	6.07 (0.05)	2.7×10^{-8}	0.15
	1	2	0	10.12 (0.07)		
	1	1	0	5.91 (0.04)	1.7×10^{-8}	0.20
	1	2	0	10.33 (0.05)		

Table 3 Thermodynamic parameters

System	l	p	q	$\Delta H°$/kJ mol $^{-1}$	$\Delta S°$/J K $^{-1}$ mol $^{-1}$
Ino H^+	0	1	1	−34.7 (1.0)	47.3
IMP–H^+	0	1	1	−37.5 (2.8)	48.2
GMP–H^+	0	1	1	−56.2 (4.6)	−8.0
Pd(tmen)–OH^-	1	0	−1	35.0 (2.9)	7.2
	1	0	−2	96.3 (9.3)	32.8
Pd(teen)–OH^-	1	0	−1	33.7 (3.3)	16.6
	1	0	−2	41.8 (15.1)	−1.3
Pd(tmen)–Ino	1	1	0	17.0 (8.4)	18.1
	1	2	0	−10.7 (23.8)	143
Pd(tmen)–IMP	1	1	0	−73.1 (27.1)	−162
	1	2	0	−62.4 (11.6)	−21.7
Pd(tmen)–GMP	1	1	0	−11.8 (8.3)	39.5
	1	2	0	−14.8 (10.9)	107
Pd(teen)–Ino	1	1	0	30.6 (6.5)	214
	1	2	0	35.6 (19.1)	322
Pd(teen)–IMP	1	1	0	−88.6 (8.6)	−211
	1	2	0	−2.09 (21.6)	163
Pd(teen)–GMP	1	1	0	−76.2 (12.7)	−176
	1	2	0	−111 (32)	−230

formation constants were found to be 6.11 ± 0.02 and 9.76 ± 0.03 for inosine, 4.81 ± 0.07 and 9.44 ± 0.02 for inosine 5′-monophosphate, and 4.86 ± 0.08 and 8.21 ± 0.10 for guanosine, respectively. These values are slightly higher than those reported in Table 1 for the complex-formation equilibria starting with the dichloro complex. This difference can be related to the role of mixed chloro–aqua complexes present in solution when the dichloro complex is used as starting material. However, the magnitude of the difference is such that the influence of the chloride concentration at the level introduced when the dichloro complex is used is rather small and does not affect the overall interpretation of the data.

The values obtained for the thermodynamic parameters $\Delta H°$ and $\Delta S°$ associated with protonation of the DNA constituents and complex formation with the Pd(amine) species were calculated from the temperature dependence of the data in Table 1 in the usual way, and are reported in Table 3. These data can be employed to extrapolate the equilibrium constants to other temperatures. The main conclusions from the data can be summarized as follows. (*a*) The protonation reactions of inosine 5′-monophosphate and guanosine 5′-monophosphate are more exothermic than those of inosine. This may be explained on the basis of different coloumbic forces operating between the ions; IMP and GMP are trinegatively charged ions, whereas inosine is a mononegatively charged ion under the conditions selected. (*b*) The complex formation of IMP and GMP with the Pd(amine) species is exothermic, whereas the reaction between the inosine anion or OH^- is endothermic. This is again ascribed to the high negative charge on IMP and GMP.

Conclusion

The results of this study have demonstrated the difficulties encountered when thermodynamic data, obtained directly from potentiometric and indirectly from kinetic measurements, are correlated. The pH dependence of the binding site on the DNA constituent and the acidity range selected for the experimental measurements can account for the apparent discrepancies. In addition, the effect of free chloride drastically influences the speciation of the complex ions in solution and controls the thermodynamic and kinetic parameters. The lability of the model aqua complexes is also controlled by pH since this determines the formation of inert hydroxo complexes. On the basis of the results presented it is difficult to predict the nature of the complex species that will be formed under biological conditions, which will be controlled by pH and the free chloride concentration. Slight differences in especially the pH of healthy and tumour cells will strongly affect the nature of the reactive species and the preferred binding site of the DNA constituent.

Acknowledgements

The authors gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft, the Volkswagen-Stiftung and Deutsche Akademische Austauschdienst. M. S. thanks the Department of Chemistry, University of Cairo, for leave.

References

1 H. Hohmann, B. Hellquist and R. van Eldik, *Inorg. Chem.*, 1992, **31**, 345.
2 H. Hohmann, B. Hellquist and R. van Eldik, *Inorg. Chem.*, 1992, **31**, 1090.
3 M. Shoukry, H. Hohmann and R. van Eldik, *Inorg. Chim. Acta*, 1992, **198–200**, 187.
4 S. Suvachittanont, H. Hohmann, R. van Eldik and J. Reedijk, *Inorg. Chem.*, 1993, **32**, 4544.
5 S. Suvachittanont and R. van Eldik, *Inorg. Chem.*, 1994, **33**, 895.

6 S. Suvachittanont and R. van Eldik, *J. Chem. Soc., Dalton Trans.*, 1995, 2027.
7 F. F. Prinsloo, J. J. Pienaar and R. van Eldik, *J. Chem. Soc., Dalton Trans.*, 1995, 3581.
8 M. Kotowski and R. van Eldik, *Inorg. Chem.*, 1984, **23**, 3310.
9 H. Hohmann and R. van Eldik, *Inorg. Chim. Acta*, 1990, **174**, 87.
10 H. Hohmann, B. Hellquist and R. van Eldik, *Inorg. Chim. Acta*, 1991, **188**, 25.
11 T. Rau and R. van Eldik, in *Metal Ions in Biological Systems*, eds. H. Sigel and A. Sigel, Marcel Dekker, New York, 1996, **32**, 339.
12 H. Sigel, S. S. Massoud and N. A. Corfu, *J. Am. Chem. Soc.*, 1994, **116**, 2958.
13 R. G. Bates, *Determination of pH – Theory and Practice*, 2nd edn., Wiley-Interscience, New York, 1975.
14 OLIS Inc., Bogart, GA, 1993.
15 M. M. Shoukry, W. M. Hosny and M. M. Khalil, *Transition Met. Chem.*, 1995, **20**, 252.
16 P. Gans, A. Sabatini and A. Vacca, *Inorg. Chim. Acta*, 1976, **18**, 237.
17 L. Pettit, University of Leeds, personal communication.
18 H. Lönnberg and P. Vihanto, *Inorg. Chim. Acta*, 1981, **56**, 157.
19 R. B. Martin and Y. H. Mariam, *Metal Ions Biol. Syst.*, 1979, **8**, 57.
20 R. W. Gellert and R. Bau, *Metal Ions Biol. Syst.*, 1979, **8**, 1.
21 B. Noszal, V. Scheller-Krattiger and R. B. Martin, *J. Am. Chem. Soc.*, 1982, **104**, 1078.

Received 19*th February* 1996; *Paper* 6/01203E