# The Hydrolysis Products of *cis*-Dichlorodiammineplatinum(II) 3. Hydrolysis Kinetics at Physiological pH

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## Abstract

The rate of hydrolysis of *cis*-$PtCl_2(NH_3)_2$ has been measured in non-buffered aqueous solution ($I$ = 0.2 M $NaClO_4$; $T$ = 45.0 °C) at constant pH over the range pH = 4.0–8.5 using a combined pH-stat/spectrophotometric technique. The hydrolysis rate at pH = 7.4 ($T$ = 45.0 °C) was also determined with $[Cl^-]$ = 0.0–0.1 M ($I$ = 0.2 M; NaCl, $NaClO_4$). At the end of each kinetic run, the resulting solution was made 0.2 M in $Cl^-$ by the addition of NaCl and the reverse (anation) reaction was monitored, again under constant pH conditions. A knowledge of the complete pH-rate profile, with and without added chloride ion, allows current models for the hydrolysis of *cis*-$PtCl_2(NH_3)_2$ under physiological conditions, to be tested. *cis*-$Pt(NH_3)_2(OH_2)_2^{2+}$ is considered to be the least important of all the potential hydrolysis products available to bind to replicating DNA at pH = 7.4.

## Introduction

*cis*-$PtCl_2(NH_3)_2$ is used extensively in antitumor cancer therapy [1]. We have previously measured the hydrolysis kinetics of *cis*-$PtCl_2(NH_3)_2$ in both acidic [2] and basic [3] solution. Table 1 summarises the characteristic features, the most important being that in acid solution an equilibrium is established (eqn. (1)), but in basic solution, irreversible hydrolysis occurs (eqn. (3)) and both chloro ligands are lost.

$$cis\text{-}PtCl_2(NH_3)_2 + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} cis\text{-}PtCl(NH_3)_2(OH_2)^+ + Cl^- \quad (1)$$

$$cis\text{-}PtCl(NH_3)_2(OH_2)^+ + H_2O \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} cis\text{-}Pt(NH_3)_2(OH_2)_2^{2+} + Cl^- \quad (2)$$

$$cis\text{-}PtCl_2(NH_3)_2 + 2OH^- \xrightarrow{k_3} cis\text{-}Pt(OH)_2(NH_3)_2 + 2Cl^- \quad (3)$$

In acid solution, reaction (1) does not proceed further (eqn. (2)) to produce significant amounts of *cis*-$Pt(NH_3)_2(OH_2)_2^{2+}$, as the small value for the equilibrium constant associated with eqn. (2) ($K_2$ = $2.7 \times 10^{-4}$ at 25 °C [3]) effectively prevents further chloride ion release. The forward rate constants for eqns. (1), (2) and (3) are in the ratio of 1.00:0.37:0.20 respectively [2, 3] (see Scheme 1).

A second important feature is that the extent of acid hydrolysis, according to eqns. (1) and (2), is chloride ion dependent, whereas if hydrolysis takes place according to eqn. (3), the reaction proceeds to completion independent of the chloride ion concentration [3].

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TABLE 1. Comparison of hydrolysis data for *cis*-$PtCl_2(NH_3)_2$ in acidic and basic solution [2, 3]

| | Kinetic parameters 25 °C ($\mu$ = 0.1 M) | Final products | Comments |
|---|---|---|---|
| Acid (0.1 M $HClO_4$) | $k_1 = 7.56 \times 10^{-5}$ s$^{-1}$<br>$\Delta H^{\ddagger} = 73.7$ kJ mol$^{-1}$<br>$\Delta S^{\ddagger} = -76.5$ J K$^{-1}$ mol$^{-1}$ | Equilibrium mixture of *cis*-$PtCl_2(NH_3)_2$, *cis*-$PtCl(NH_3)_2(OH_2)_2^+$ and $Cl^-$ | Rate independent of $[H^+]$ and ionic strength: extent of reaction Suppressed by added $Cl^-$ |
| Base (0.1 M NaOH) | $k_3 = 1.90 \times 10^{-5}$ s$^{-1}$<br>$\Delta H^{\ddagger} = 84.4$ kJ mol$^{-1}$<br>$\Delta S^{\ddagger} = -52$ J K$^{-1}$ mol$^{-1}$ | *cis*-$Pt(OH)_2(NH_3)_2$ and 2 $Cl^-$ | Rate independent of $[OH^-]$, ionic strength and $[Cl^-]$ |

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It is thus important to determine the pH-rate profile in the range pH = 4–9, and this is the basis of the present publication.

## Experimental

*cis*-$PtCl_2(NH_3)_2$ was purchased from Strem Chemical Company and used as supplied. All other reagents were AR quality or the best reagent grade available. A Radiometer pH-stat (TTTlc) coupled to a Radiometer Titrigraph (SBR2) with the appropriate glass (G202C) and calomel (TS-1) electrodes was used for all pH measurements. The instrument was calibrated with 0.01 M borax buffer (pH = 9.038 at 45.0 °C). A Varian DMS100 recording spectrophotometer was used to record the UV (345–220 nm) spectra of the reacting solutions.

*Kinetic Measurements at Constant pH*

A total of 50 mg of *cis*-$PtCl_2(NH_3)_2$ was dissolved in *c.* 5 ml of DMF, and this solution was added to 70 ml of 0.2 M $NaClO_4$ solution ($NaClO_4$; NaCl, $I$ = 0.2 M when $Cl^-$ variation studies were performed) in the temperature controlled (45.0 ± 0.1 °C) reaction vessel of the Radiometer pH-stat while this solution was being peristaltically pumped (100 ml/min) through a 2.00 cm flow-through cell in the Varian spectrophotometer. Glass and calomel electrodes were placed in the reactant solution and the pH adjusted to just above the set pH by the addition of dilute acid or alkali. As the hydrolysis proceeds (above pH = 5), the pH drops and when the set pH was reached, the pH-stat system was activated by the automatic addition of NaOH solution (0.05 M) through a stainless steel needle. When constant pH was satisfactorily maintained, the repeat scan/fixed wavelength modes of the spectrophotometer were activated to monitor absorbance with time. The concentrations are such that 6.66 ml of 0.05 M NaOH is required if two moles of $OH^-$ are used per mole of platinum(II). Thus, the proportion of NaOH for complete reaction, the rate of NaOH uptake and the rate of spectrophotometric change were measured simultaneously.

Control experiments showed the system behaved identically if the DMF was not present.

On the completion of each kinetic run, NaCl (0.877 g) was added to the reaction solution to give a [$Cl^-$] of 0.2 M and the repeat scan mode of the spectrophotometer was reactivated. Constant pH was maintained by periodic manual addition of 0.1 M $HClO_4$. Under these conditions, aqua ligands coordinated to Pt(II) are fairly rapidly replaced by $Cl^-$. The results are summarised in Table 2.

## Results and Discussion

The generally accepted mechanism for the interaction of *cis*-$PtCl_2(NH_3)_2$ with the target DNA molecule is as follows [4]. *cis*-$PtCl_2(NH_3)_2$, stabilised in saline solution, enters the blood stream under high hydration therapy [5]. The relatively high chloride ion concentration (~100 mM) in blood plasma prevents hydrolysis of the chloro ligands and the neutral molecule passes through the cell wall. Once inside the cell, hydrolysis according to eqn. (1) can now proceed as the background chloride ion concentration has dropped to ~4 mM. The positively charged (chloro)(aqua) product is the most likely labile complex for donor groups in the DNA target,

TABLE 2. Rate data for the hydrolysis of *cis*-$PtCl_2(NH_3)_2$ at various fixed pH in unbuffered media ($I$ = 0.2 M, $NaClO_4$; $T$ = 45.0 °C)

| Set pH | $OH^-$ uptake (mole/mole Pt(II)) | $10^4 \times k_{obs}$[a] ($s^{-1}$) | Isosbestic points (nm) | Chloride ion uptake[b] $10^4 \times k_{Cl}$ ($s^{-1}$) | Isosbestic points[c] (nm) |
|---|---|---|---|---|---|
| 2.0 | 0.00 | 3.80 ± 0.35 | 282 | [d] | [d] |
| 4.0 | 0.00 | 3.71 ± 0.25 | 283 | 83.5 ± 4 | [d] |
| 5.5 | 0.00 | 3.57 ± 0.44 | 277 then 289 | 68.2 ± 6 | 281 then 289 |
| 6.0 | 0.37 | 3.37 ± 0.33 | 277 then 285 | 45.5 ± 3 | 277 then 287 |
| 6.5 | 0.68 | 3.13 ± 0.17 | 274 then 284 | 15.9 ± 2 | 274 then 284 |
| 6.8 | 0.62 | 2.50 ± 0.19 | 272 then 280 | 12.9 ± 0.6 | 273 then 285 |
| 7.0 | 0.77 | 2.37 ± 0.11 | 271 then 279 | 10.9 ± 0.7 | 272 then 285 |
| 7.2 | 0.90 | 2.04 ± 0.25 | 277 | 9.43 ± 0.5 | 273 |
| 7.5 | 1.10 | 1.97 ± 0.15 | 276 | 7.67 ± 0.4 | 271 |
| 8.0 | 1.28 | 1.79 ± 0.16 | 276 | 5.48 ± 0.4 | 270 |
| 8.5 | 1.71 | 1.87 ± 0.25 | 276 | 4.06 ± 0.4 | 273 |
| 12.0 | 2.0 | 1.84 | 276 | no reaction | |

[a]Observed first order rate constant for the overall forward reaction. [b]Final [$Cl^-$] = 0.2 M. $k_{Cl}$ is proportional to the total *cis*-$PtX(NH_3)_2(OH_2)^+$ concentration [X = Cl, OH]. [c]For the chloride ion anation reaction. [d]The reaction was too fast to obtain these accurately.

but many workers investigating DNA or model DNA interacting systems have used the di(aqua) as the labile platinum species [6–15]. There is, however, recent evidence for direct attack by GMP on *cis*-$PtCl_2(NH_3)_2$ [16] although such changes have been observed previously [7] and given a different interpretation.

The above model [4] is based on rate constant, equilibrium constant and hydrolysis product data, accumulated in the pH = 0–6.5 region [2–4, 17–33] and is dominated by the chloride ion dependent equilibria inherent in eqns. (1) and (2).

Rather fewer studies on the hydrolysis of *cis*-$PtCl_2(NH_3)_2$ have been made in alkaline solution (pH > 9). Under these conditions, both chloride ions are lost irreversibly [3, 17, 21, 22, 34] (eqn. (3)) and the rate is independent of the background chloride ion concentration, the hydroxide ion concentration or the ionic strength [3].

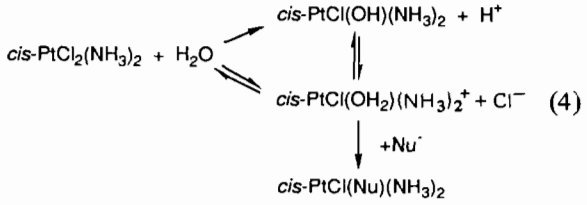
If the base hydrolysis process were to operate at pH = 7.4 (the pH of blood plasma), then an entirely different model could be proposed for the mechanism of the *cis*-$PtCl_2(NH_3)_2$–DNA interaction.

In this regime, the chloride ion hydrolysis would proceed irreversibly via eqn. (3), independent of background chloride ion concentrations. The pH and chloride ion concentrations are such that the final product would be a mixture of *cis*-$Pt(OH)(NH_3)_2(OH_2)^+$, *cis*-$PtCl(OH)(NH_3)_2$ and *cis*-$Pt(OH)_2(NH_3)_2$. The di(hydroxo) and (chloro)(hydroxo) are relatively inert to substitution, but capable of cell wall penetration in the neutral form, whereas the (hydroxo)(aqua) or (chloro)(aqua) ions would be the active reagent for attack by polar donor groups in the DNA target molecule. The rapid protolytic equilibria between the (chloro)(hydroxo) and the (chloro)(aqua) would allow facile transport of Pt(II) species to suitable DNA sites.

To sustain this alternative model requires a measurement of the rate and the extent of the hydrolysis of *cis*-$PtCl_2(NH_3)_2$ in the pH region 4.5–8.5 both with and without added chloride ion. This has not been attempted previously and is not a trivial problem as there are a number of complicating features.

The conventional technique to control pH in the 6.5–8.0 region is by use of buffers [35]. Many of these contain nitrogen, sulphur or oxygen groups that are potential donor atoms for the Pt(II) center. If the buffer compounds are acting as nucleophiles [49], they could be markedly affecting the reaction kinetics [29, 32] and the buffer substrate will be coordinated to the Pt(II) at the end of the reaction.

Our preliminary results (not described here) indicated that all buffer systems we have tried (MES, HEPES, TRIS, BISTRIS and BICINE) are interacting with the Pt(II) hydrolysis products (eqn. (4)).




(4)

$Nu^-$ is the buffer nucleophile.

A non-buffer procedure to control the pH of a reaction proceeding with a pH change is by use of a pH-stat [36, 37]. This is an excellent technique for reactions proceeding with $OH^-$ consumption as, apart from electrolytes added to control the ionic strength, $OH^-$ is the only nucleophile present. There are three disadvantages: (i) the set pH must be greater than the $pK_a$ for the hydroxo/aqua equilibrium of the product [38, 39]; (ii) the conversion of pH to $[OH^-]$ is subject to the inclusion of non-specific parameters (e.g. $Na^+$ corrections) [39]; and (iii) the only measureable parameter is the rate of $OH^-$ uptake and consequently the nature of the products can be misinterpreted.

The latter disadvantage can be overcome to a considerable extent by simultaneously recording the absorption spectrum while maintaining constant pH [40, 41]. This technique gives both the change in absorption spectrum and the extent of $OH^-$ uptake at the same time, under non-buffered, constant pH conditions, and is the method employed here.

Another major problem that has been alluded to previously, is one of product identification. The hydrolysis products of *cis*-$PtCl_2(NH_3)_2$ are known to form dimers $[Pt(NH_3)_2OH]_2^{2+}$ [42] and trimers $[Pt(NH_3)_2OH]_3^{3+}$ [43], and there have been suggestions [44, 45] that these are responsible for the toxic properties of the drug. The absorption spectra of these oligomers have been reported [46, 50] but the data are not in agreement* and definitive spectral information (apart from $^{195}Pt$ NMR [4, 47, 48]) is not readily available. ($\epsilon_{330}$ values for $[Pt(NH_3)_2(OH)_2]$, $Pt(OH)(NH_3)(OH_2)^+$, $[Pt(NH_3)_2(OH)]_2^{2+}$ and $[Pt(NH_3)_2(OH)]_3^{3+}$ are about 25 [2]; 48 [50]; 75 [46]; 71 [50]; and 150 [46]; 14* [50] $M^{-1}$ $cm^{-1}$, respectively.)

Polymers of this type are more likely to be formed in concentrated solution as the rate of formation is proportional to the square of the monomer concentration [4]. We find no evidence for species with high $\epsilon_{330}$ values in our pH-stat studies and the product formed from *cis*-$PtCl_2(NH_3)_2$ in base, (*cis*-$Pt(OH)_2(NH_3)_2$ in 0.01 M NaOH [2]) passes quantitatively through a cation exchange column in the $Na^+$ form, as expected for an uncharged species. Spectral changes reported for *cis*-$Pt(NH_3)_2(OH_2)_2^{2+}$

*We suspect a typographical error may be responsible for the discrepancy.

in the pH = 4–5 region, in the presence of buffers, have been attributed to dimer formation. Surprisingly, the observed rate constants were independent of pH and changes in rate with change in buffer medium were attributed to ionic strength differences [46]. As the di(aqua) plus buffer nucleophile reaction [49] was not considered a kinetically important event, we are not convinced that the observed spectral changes are entirely due to dimer formation.

One other interesting observation is that the dimer and trimer are unstable (hours) at room temperature in 0.15–0.4 M $Cl^-$, producing *cis*-$PtCl_2(NH_3)_2$ and *cis*-$PtCl(NH_3)_2(OH_2)^+$ [48].

All we can say, with regard to our own investigations, is that we have no evidence for the production of oligomers under the conditions of our experiments.

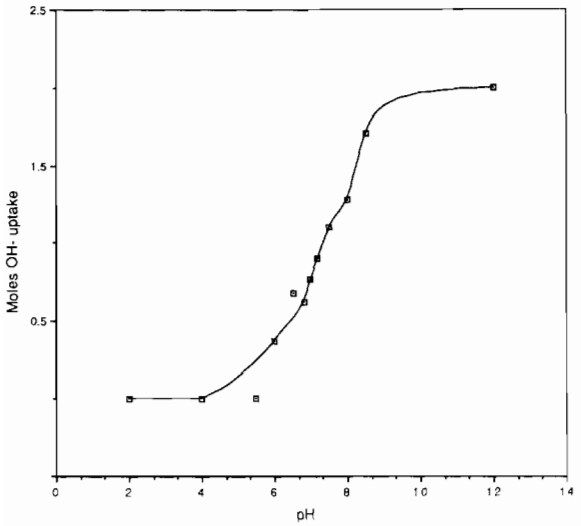



Fig. 1. Mole of $OH^-$ uptake/mole of Pt(II) at 45.0 °C ($I$ = 0.2 M, $NaClO_4$).

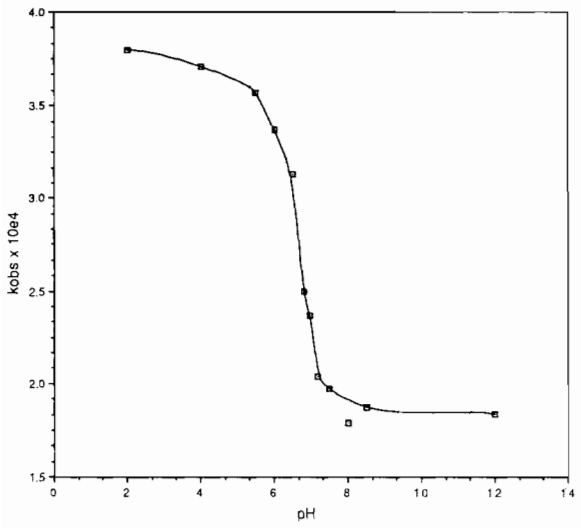



Fig. 2. $10^4 \times k_{obs}$ vs. pH at 45.0 °C and $I$ = 0.2 M ($NaClO_4$).

The hydrolysis of *cis*-$PtCl_2(NH_3)_2$ at constant, unbuffered pH, proceeds with $OH^-$ uptake that increases as the fixed pH increases. At pH = 4, there is negligible $OH^-$ uptake. The spectrophotometric scans and observed rate constant indicate normal acid hydrolysis [2]. As the fixed pH is increased the amount of $OH^-$ consumed also increases (Table 2, Fig. 1) and there is a gradual decrease in the hydrolysis rate (Fig. 2). At pH = 6.55, 0.5 mol of $OH^-$ is consumed, corresponding to the equilibrium system (5).

$$cis\text{-}PtCl_2(NH_3)_2 + 2H_2O \rightleftharpoons \begin{matrix} cis\text{-}PtCl(OH)(NH_3)_2 + H^+ \\ \Vert\ pK_a = 6.85\ [51] \\ cis\text{-}PtCl(NH_3)_2(OH_2)^+ + Cl^- \end{matrix} \quad (5)$$

Again, at pH = 8.0, 1.5 mol of $OH^-$ is consumed, as the new equilibrium system (6) is established.

$$cis\text{-}PtCl_2(NH_3)_2 + 2H_2O \rightleftharpoons \begin{matrix} cis\text{-}Pt(OH)_2(NH_3)_2 + 2H^+ \\ \Vert\ pK_a = 7.87\ [51] \\ cis\text{-}Pt(OH)(NH_3)_2(OH_2)^+ + 2Cl^- \end{matrix} \quad (6)$$

At pH > 9.0, complete base hydrolysis is taking place and 2 mol of $OH^-$ are consumed per mole of Pt(II) (eqn. (3)). Once the hydrolysis reaction is complete, the extent of chloride uptake at any fixed pH depends on the amount of $Pt-OH_2^{n+}$ species present. Consequently, the pattern observed is the reverse of the $OH^-$ uptake although the amount of acid uptake was not quantitatively established. At pH > 8 no chloride uptake is observed, but rapid and extensive chloride anation takes place at pH 4–5. Between these extremes, the pH increases as chloride uptake occurs (the reverse of eqns. (5) and (6)) and the reaction stops prematurely unless constant pH is maintained by the addition of acid.

During the course of the forward reaction at fixed pH in the range 4.0–8.5 the spectrophotometric changes indicate that as the pH increases, the reaction, which was originally monophasic, becomes biphasic (i.e. two consecutive first order reactions) and then reverts to a monophasic system again. As the set pH is changed under biphasic conditions, the position of the isosbestic points for the two reactions also changes. Where the forward reaction was biphasic, the addition of chloride ion also resulted in a biphasic system, with the generated isosbestic points mirroring the hydrolysis reaction (Table 2).

At set pH values of 5.5–7.2, the hydrolysis of *cis*-$PtCl_2(NH_3)_2$ will proceed to give *cis*-$Pt(OH)Cl(NH_3)_2$ and *cis*-$PtCl(NH_3)_2(OH_2)^+$ in a fixed ratio, and with a fixed isosbestic point. The ratio will

depend on the $K_a$ for the (chloro)(aqua)–(chloro)-(hydroxo) equilibrium ($pK_a$ = 6.85 at 4 °C [51]) and the set pH. The position of the isosbestic point will also depend on the value of the ratio. *cis*-$PtCl(NH_3)_2(OH_2)^+$ will not hydrolyse spontaneously due to an unfavourable chloride ion dependent equilibrium [3], but *cis*-$PtCl(OH)(NH_3)_2$ can hydrolyse (at a rate about 5 × slower than the first hydrolysis step [3]) independent of the chloride ion concentration. Reduction of the *cis*-$PtCl(OH)(NH_3)_2$ concentration (by hydrolysis to *cis*-$Pt(OH)(NH_3)_2(OH)_2^+$) will result in a reduction of the *cis*-$PtCl(NH_3)_2(OH_2)^+$ concentration as the equilibrium ratio must be maintained at a value determined by the set pH. Eventually the released chloride ion will establish an equilibrium with the *cis*-$Pt(OH)(NH_3)_2(OH_2)^+$ and further chloride ion release from *cis*-$PtCl(OH)(NH_3)_2$ will stop, but it is this second step that maintains the second isosbestic point. The final solution contains a mixture of *cis*-$PtCl(NH_3)_2(OH_2)^+$, *cis*-$PtCl(OH)(NH_3)_2$, *cis*-$Pt(OH)(NH_3)_2(OH_2)^+$ and $Cl^-$ ion, with the concentration of the former becoming smaller as the set pH is increased. Addition of chloride ion to this mixture results in the anation of the $Pt–OH_2^{n+}$ species, reversing the hydrolysis steps and producing a final mixture of *cis*-$PtCl_2(NH_3)_2$ and *cis*-$PtCl(OH)(NH_3)_2$.

As the set pH is further increased (7.2–8.5), the (hydroxo)(aqua)–di(hydroxo) equilibrium becomes dominant ($pK_a$ = 7.87 at 25 °C [51]) and the reaction rate slows.

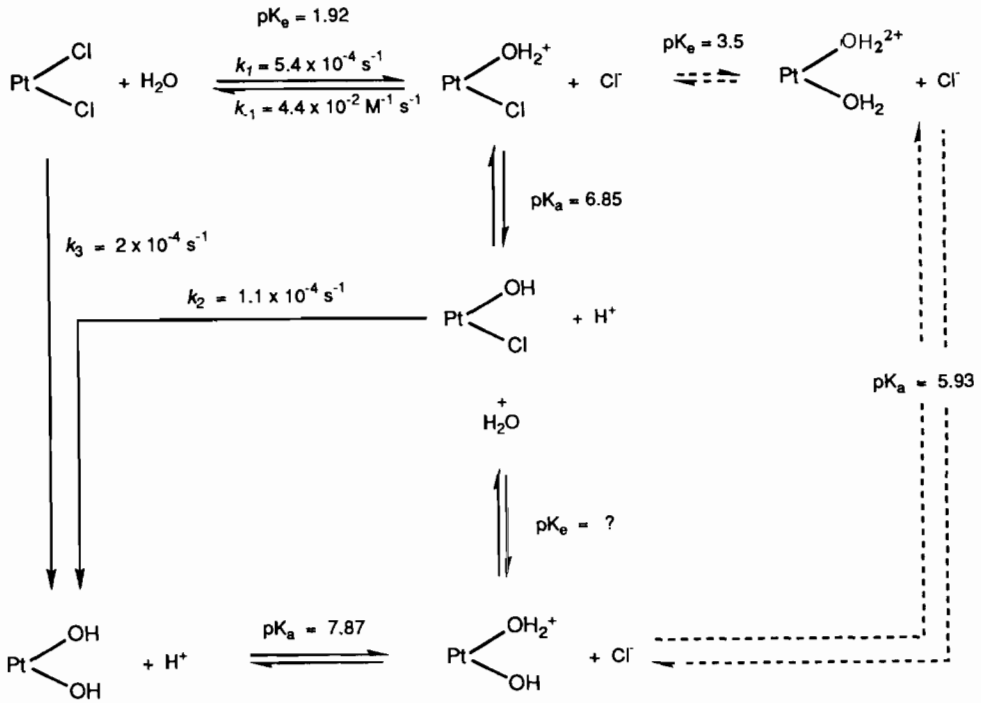
In this region, the rate is controlled by chloride release from the (chloro)(hydroxo) [the slowest step] to give an equilibrium mixture of (chloro)-(hydroxo), (hydroxo)(aqua) and di(hydroxo) with the proportion of di(hydroxo) increasing with increasing set pH. Addition of chloride ion again results in anation of all $Pt–OH_2^{n+}$ species and as these are dominated by *cis*-$Pt(OH)(NH_3)_2OH_2^+$, the final anated mixture consists of *cis*-$PtCl(OH)(NH_3)_2$ and *cis*-$Pt(OH)_2(NH_3)_2$. As the fixed pH is increased, the amount of *cis*-$Pt(OH)(NH_3)_2(OH_2)^+$ in the final mixture decreases and the extent of chloride ion uptake also decreases.

A summary of the hydrolysis processes in the absence of added chloride ion is shown in Scheme 1 and the system would be complete if rate ($k_r$) and equilibrium constants ($K_{eq} = k_f/k_r$) were known for reaction (7).

$$cis\text{-}PtCl(OH)(NH_3)_2 \underset{k_r}{\overset{k_f}{\rightleftharpoons}} cis\text{-}Pt(OH)(NH_3)_2(OH_2)_2^+ + Cl^- \quad (7)$$

We are currently exploring techniques to make such measurements, with due cognisance of the possibility of competitive dimerisation of the (hydroxo)-(aqua).

A series of kinetic runs were performed at pH = 7.4, $T$ = 45.0 °C, $[Pt(II)]_t$ = 2.2 × $10^{-3}$ M with varying amounts of $Cl^-$ present ($I$ = 0.2 M; NaCl, $NaClO_4$) (Table 3). At $[Cl^-]$ = 0, and pH = 7.4 the




Scheme 1. $T$ = 45.0 °C, data from refs. 2, 3, 51.

TABLE 3. Rate data for the hydrolysis of *cis*-$PtCl_2(NH_3)_2$ at pH = 7.4 with variable chloride ion concentration ($I$ = 0.2 M, $NaClO_4$, NaCl; $T$ = 45 °C, [*cis*-$PtCl_2(NH_3)_2$] = 2.2 mM)

| $[Cl^-]_i$ (M) | $OH^-$ uptake (mole/mole Pt(II)) | | $10^4 \times k_{obs}$[a] ($s^{-1}$) | | $Cl^-$ uptake[b] $10^4 \times k_{Cl}$ ($s^{-1}$) | |
|---|---|---|---|---|---|---|
| | obs. | calc.[c] | obs. | calc.[d] | obs. | calc.[e] |
| 0.00 | 1.08 | 1.08 | 1.97 ± 0.15 | 1.97 | 7.95 ± 0.4 | 7.95 |
| 0.010 | 0.68 | 1.00 | 2.12 ± 0.10 | 2.15 | 7.47 ± 0.4 | 7.65 |
| 0.025 | 0.71 | 0.90 | 2.48 ± 0.12 | 2.43 | 6.16 ± 0.4 | 7.20 |
| 0.050 | 0.75 | 0.72 | 2.61 ± 0.19 | 2.90 | 7.04 ± 1 | 6.44 |
| 0.075 | 0.38 | 0.53 | 3.32 ± 0.59 | 3.34 | 5.86 ± 1 | 5.68 |
| 0.100 | 0.35 | 0.35 | 4.23 ± 0.88 | 3.80 | 4.93 ± 0.3 | 4.93 |

[a]First order rate constant for the overall forward reaction. [b]Final $[Cl^-]$ = 0.2 M. The rate is proportional to the amount of *cis*-$PtCl(NH_3)_2(OH_2)^+$ at equilibrium after completion of the forward reaction. [c]Calculated from the linear relationship: mole $OH^-$ uptake/mole Pt(II) = 1.08–7.3 $[Cl^-]_i$ ($T$ = 45.0 °C, pH = 7.4, $I$ = 0.2 M). [d]Calculated from the linear relationship: $10^4 \times k_{obs}$ = 18.3 $[Cl^-]_i$ + 1.97 (45.0 °C, pH = 7.4, $I$ = 0.2 M). [e]Calculated from the linear relationship: $10^4 \times k_{Cl}$ = 7.95–30.2 $[Cl^-]_i$ (45.0 °C, pH = 7.4, $[Cl^-]$ = 0.2 M, $I$ = 0.2–0.3).

hydrolysis reaction is monophasic (see earlier), an equilibrium mixture of (chloro)(hydroxo) and (hydroxo)(aqua) is produced in about a 60:40 ratio ($pK_a$ for the (chloro)(aqua)–(chloro)(hydroxo) = 6.85 [51]), and 1 mol of $OH^-$ is consumed/mole of Pt(II). The increase in $k_{obs}$ with increasing $[Cl^-]$ is due to the fact that the system is proceeding to equilibrium and simple first order kinetics are not strictly applicable to eqn. (1). As the $[Cl^-]$ increases the amount of $OH^-$ uptake decreases, the extent of the reaction decreases, but the ratio of (chloro)(aqua) to (chloro)(hydroxo) remains constant as the pH is constant. Naturally, the rate of reverse reaction (with excess $Cl^-$) decreases with increasing initial $[Cl^-]$ because the amount of (chloro)(aqua) at equilibrium is decreasing. At $[Cl^-]_{initial}$ of 0.1 M, the final equilibrium ratio of products (pH = 7.4, $T$ = 45.0 °C) is about 50% di(chloro), 30% (chloro)(hydroxo) and 20% (chloro)(aqua). This result is obtained from the self consistency of the following features:

(a) the final visible absorption spectra of the hydrolysis reaction

(b) the uptake of 0.37 mol of $OH^-$ per mole of Pt(II)

(c) the rate of chloride anation

(d) the final visible absorption spectra of the anation reaction

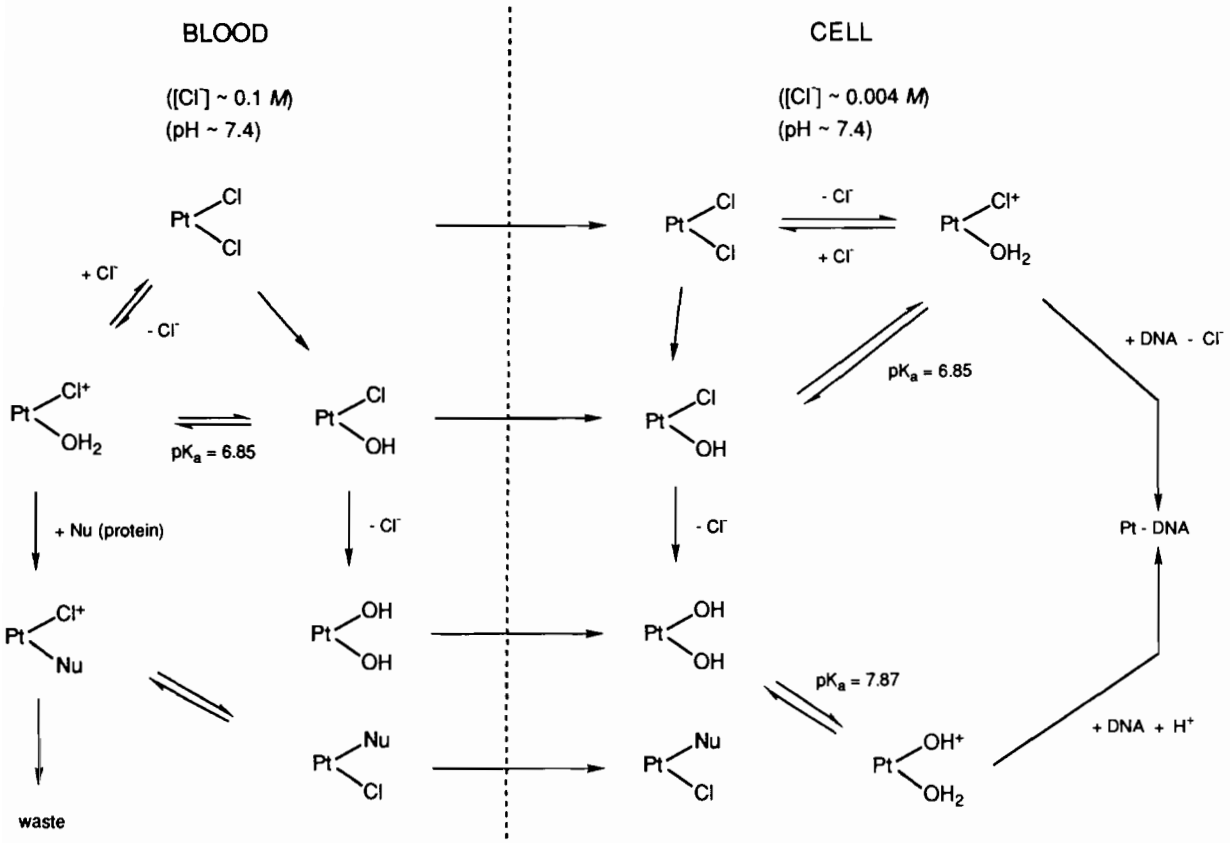
(e) the $pK_a$ value of 6.85 [51] for the (chloro)(aqua)–(chloro)(hydroxo) equilibrium. We estimate that this equilibrium mixture would be produced in about 6 h at 37 °C under the above conditions of pH and $[Cl^-]_i$ and as the $[Pt(II)]_{initial}$ drops, the proportion of dichloro still present at equilibrium will increase.

Hydrolysis schemes for the reaction of *cis*-$PtCl_2(NH_3)_2$ in the biological milieu are frequently reported [52–58] but the relative importance of any particular species is not normally highlighted.

One of the most successful attempts is that of LeRoy *et al.* [53] who analyse their hydrolysis scheme in terms of the equilibrium constant data known at that time (1979). They conclude that at pH = 7.5 and $T$ = 37 °C with $[Cl^-]$ = 0.1 M and $[Pt]_t = 10^{-6}$ M the dichloro (>83%), (chloro)(aqua) (4%) and (hydroxo)(aqua) (12%) species predominate and that the di(hydroxo), di(aqua) and (hydroxo)(aqua) species are present at the <1% level. Using more modern estimates for the equilibrium constants [2, 3, 51] we calculate 68%, 7% and 24% for the above, most predominate species, respectively, at pH = 7.5, $[Cl^-]$ = 0.1 M, $[Pt]_t = 10^{-3}$ M and $T$ = 25 °C.

In cytoplasm, the $[Cl^-]$ drops to ~4 mM and LeRoy *et al.* [53] calculate 31% dichloro, 28% (chloro)(aqua), 32% (hydroxo)(chloro), and 7% (hydroxo)(aqua) with the di(hydroxo) and di(aqua) at the <1% level. Although quite different conclusions are reached by Roos [54] (47% di(aqua) in cytoplasm medium), we agree with the analysis of LeRoy *et al.* and suggest that *cis*-$Pt(NH_3)_2(OH_2)_2^{2+}$ is one of the least likely Pt(II) species, at physiological pH, for attack by donor atoms on DNA. Consequently, while studies using *cis*-$Pt(NH_3)_2(OH_2)_2^{2+}$ plus nucleophiles as models for the *cis*-DDP/DNA interaction may provide an order of nucleophilic ability, they probably have little relevance to processes taking place at the site of the replicating cancer cells.

Our observations that buffer nucleophiles interact readily with *cis*-$PtCl_2(NH_3)_2$ hydrolysis products make the models for Pt(II) transport in biological systems (such as blood plasma) extremely difficult to design.




Scheme 2. $NH_3$ ligands have been omitted.

If *cis*-$PtCl_2(NH_3)_2$ were to hydrolyse in the blood stream at pH = 7.4 via eqns. (5) and (6), then blood serum albumin or other plasma protein would be the most likely nucleophiles for the *cis*-$Pt(X)(NH_3)_2(OH_2)^+$ (X = OH, Cl) species. This is equivalent to the buffer nucleophile situation. If the anating ligands are neutral, the resulting complex will remain charged and be effectively wasted, as transport of charged species across cell membranes is less likely than transport of neutral complexes. The most likely neutral species are the unreacted parent, *cis*-$PtCl_2(NH_3)_2$, and *cis*-$Pt(OH)(Cl)(NH_3)_2$ although there could also be traces of *cis*-$Pt(OH)_2(NH_3)_2$. A somewhat speculative model is given in Scheme 2.

## Conclusions

Our results for the hydrolysis of *cis*-$PtCl_2(NH_3)_2$ in 0.1 M NaCl solution at pH = 7.4, suggest that the Rosenberg model for Pt(II) transport *in vivo* may need some modification. At physiological pH and chloride ion concentration, hydrolysis of the dichloro is measurable ($t_{1/2} \sim 1$ h at 37 °C) and if no Pt(II) species are removed, an equilibrium di(chloro), (chloro)(aqua), (chloro)(hydroxo) system is produced in about 6 h (37 °C). There are now two neutral species [di(chloro) and (chloro)(hydroxo)] available for transport through the cell wall. If cell wall transfer is rapid relative to the hydrolysis rate, then the Rosenberg model is valid. However, if cell wall transfer is slow, then both the di(chloro) and (chloro)(hydroxo) would be available. It is also possible that the (chloro)(aqua) in the blood stream could be removed from the system via binding to plasma protein before cell wall transfer [via conversion to the (chloro)(hydroxo)] and this process would also upset the rapidly interconverting (chloro)(aqua)–(chloro)(hydroxo) equilibrium concentrations.

Within the cell, ($[Cl^-] \sim 4$ mM), almost complete hydrolysis of the dichloro will occur ($t_{1/2} \sim 2$ h at 37 °C) to give (at pH = 7.4) a 50:50 (chloro)(aqua)–(chloro)(hydroxo) equilibrium mixture (assuming no Pt(II) loss). Pt(II) removal can occur, however via DNA binding to the (chloro)(aqua) [13]. Thus, while we are now beginning to understand the hydrolysis behaviour of *cis*-$PtCl_2(NH_3)_2$ in aqueous solutions that model the physiological situation, we are still some way from a complete knowledge of the *in vivo* system.

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