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The degradation of carboplatin in aqueous solutions containing chloride or other selected nucleophiles

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Summary

The degradation kinetics of carboplatin in aqueous solution in the presence and absence of chloride ions were studied at elevated temperatures (43–70 °C) at pH 7.0 and at various pH values at 70 °C. Reaction rates were then extrapolated to temperatures of interest (infusion storage temperature, 'in-use' infusion temperature and body temperature) from Arrhenius plots. Additionally, the effects of pH and added chloride, thiosulphate, thiocyanate, azide and iodide on the rate of degradation of carboplatin were determined in an attempt to obtain a greater insight into the mechanism of nucleophilic substitution of carboplatin.

Introduction

The platinum co-ordination complex cisplatin (*cis*-diammine-dichloroplatinum (II)) was introduced to the clinic as a cytotoxic agent in 1972. Cisplatin is indicated in the treatment of ovarian and testicular neoplasms and has also been used with some success against tumors of the lung, bladder, cervix and head and neck (Calvert et al., 1982). Clinical experience has shown that cisplatin is highly nephrotoxic. This problem can be partially alleviated by hydration with mannitol infusions and diuresis (Penta et al., 1983), but progressive decline of both glomerular and tubular function occurs with continued treatment (Harland et al., 1984). These difficulties have been largely overcome by the development of carboplatin (*cis*-diammine-1,1-cyclobutane dicarboxylate platinum(II), **1**), a non-nephrotoxic analog of cisplatin having similar antitumor activity. (Harrap et al., 1980).

For **1** to become biologically active it is envisaged that one or both of the cyclobutane dicarboxylate ligands are replaced with water to

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produce a more reactive platinum species (Harland et al., 1984). There is also evidence that **1** may undergo nucleophilic substitution reactions with chloride ions resulting in conversion to cisplatin (Cheung et al., 1987). It has also been shown that **1** is sufficiently stable in the absence of chloride ions when prepared in aqueous solution for use in prolonged continuous infusion regimes (Sewell et al., 1987a). However, multiple agent regimes requiring the admixture of **1** with other cytotoxic agents could result in the introduction of chloride ions into the infusion, either as drug counterions or as excipients. The presence of relatively low chloride ion concentrations could be significant in continuous infusion regimes where long-term stability in aqueous solution is required (Sewell et al., 1987b).

The reaction of carboplatin with chloride ions could also have implications in vivo, especially in the extracellular fluids where the chloride ion concentration is approx. 0.1 M. It is therefore possible that chloride-substituted derivatives of **1** (including cisplatin (**5**)) could be produced in the blood (Scheme 1). Other blood nucleophiles include thiocyanate (30.7 μmol l^{-1}) and iodide (410 nmol l^{-1}). It is possible that these ions could also react with **1** by nucleophilic substitution. A phase I/II study on carboplatin administered by continuous infusion with synchronous radiotherapy is being conducted at this institution (Exeter) and the nature of platinum species in the systemic circulation and their relative radiosensitization activity is of obvious interest.

Materials and Methods

Reagents

Carboplatin was supplied by Bristol Myers Ltd (Uxbridge, U.K.) for experimental use. All other reagents were of analytical reagent grade or HPLC grade as appropriate (BDH Ltd Poole, U.K.).

Assay of carboplatin

Liquid chromatography was conducted on an isocratic system comprising: model 3000 pump, model 3000 detector, CI-10B integrator/printer plotter and Rheodyne type 7125 loop valve (LDC-Milton Roy Ltd). The LC assay for the degradation of **1** in the presence of chloride ions was developed and validated from a method which was previously shown to be stability indicating (Sewell et al., 1987b). The chromatographic conditions were: column (250 × 4.9 mm) packed with Spherisorb ODS (5 μm); mobile phase 0.02 M phosphate buffer (pH 6.5) at a flow rate of 1 ml min^{-1}. Samples were injected in duplicate (20 μl) and each group of four samples was 'bracketed' by duplicate injections of an external standard solution of **1** (0.01 mg ml^{-1}). Test solutions were diluted volumetrically with water to approximately 0.01 mg ml^{-1} **1** prior to assay. The concentration of **1** in sample solutions was determined from the mean peak height ratios of the test and external standard peaks.

To investigate the reactivity of **1** with other nucleophiles a column (250 × 4.9 mm) packed with Phenomenex Ultracarb (5 μm) was used. Samples were injected in duplicate and bracketed by injections of external standard. All the other assay conditions were identical to those described above. The linearity of carboplatin peak height with the concentration of **1** injected was established over the concentration range 0.002–0.016 mg ml^{-1}. A typical relationship between the concentration of **1** injected (x) and peak height (y) was $y = 4.59 \times 10^3 x - 0.40$ ($n = 8$, $r = 0.999$). A stock solution containing **1** (1 mg ml^{-1}) was subjected to eight replicate dilutions (1 in 100). The concentration of **1** in each final dilution was determined by LC and the relative standard deviation (RSD) for the replicate assays was 0.4% ($n = 8$).

Kinetic experiments

In general, the degradation of **1** was followed by LC for at least two half-lives of the parent compound. Unless otherwise stated, the ionic strength was adjusted to 1.0 with KNO_3 (Riley et al., 1982). The effects of temperature (43–70 ± 0.2 °C) at pH 7.0 and of pH (4.0–10.0) at 70 °C on the degradation kinetics of **1** in aqueous solution were determined in the absence and presence of 0.4 M sodium chloride. The effect of the nucleophiles chloride (0.1–0.4 M), thiosulphate (0.017–0.125 M), thiocyanate (0.15–0.5 M), azide (0.5–0.96 M) and iodide (0.06–0.11 M) on the

degradation kinetics of carboplatin in aqueous solution was also investigated at 30 °C and pH 7.0. The effect of ionic strength (0.4–2.0) was studied at pH 7.0 and 70 °C in the absence and presence of 0.4 M NaCl.

Data analysis

Pseudo first-order rate constants were obtained by least-squares linear regression analysis of the relationship relating the logarithm of the concentration of **1** remaining and time. Data were fitted to non-linear equations (pH profiles) using the program RS1 on a Compaq 384 personal computer. Computer simulations were generated with the spreadsheet program MS Works and the graphics program Cricket Graph Version 1.2 on a Macintosh IICX personal computer (Apple Computers).

Results and Discussion

Effect of chloride ion

The degradation of **1** in the presence of chloride was found to be pseudo first-order over at least two half-lives (Fig. 1). The observed pseudo first-order rate constant for the loss of **1** (obtained by least-squares linear regression) was found to be linearly (k_{obs}) related to the concentration of chloride (Fig. 2) according to Eqn 1, which has been described previously for the nucleophilic substitution of other square planar platinum(II) complexes (Bellucco et al., 1965, Riley, et al., 1982):

$$k_{obs} = k_0 + k_1 [Cl^-] \quad (1)$$

where k_0 is the rate constant for hydrolysis and k_1 is the rate constant for nucleophilic substitution by chloride.

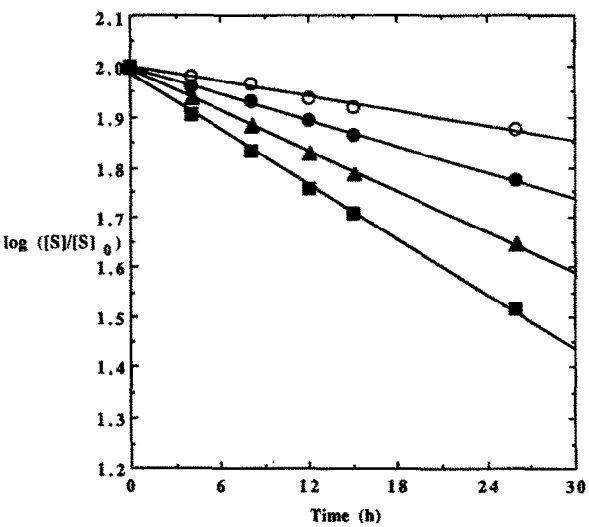


Fig. 1. Typical first-order plots for the degradation of **1** at 70 °C in the presence of various concentrations of NaCl. [Cl^-]: (□) 0, (●) 0.1 M, (▲) 0.25 M, (■) 0.4 M.

TABLE 1

Values of rate constants, k_{obs}, k_0 and k_1 (Eqn 1) for the degradation of carboplatin at pH 7.0, at various temperatures and in the presence and absence of chloride (μ = 1.0)

Temperature (°C)	[Cl^-] (M)	$k_{obs}(\times 10^3)$ (h^{-1})	$k_0(\times 10^3)$ (h^{-1})	$k_1(\times 10^2)$ ($M^{-1}\,h^{-1}$)
70 [a]	0	38.39		
	0.1	54.79	37.5	18.1
	0.25	81.53		
	0.4	110.5		
59 [a]	0	11.24		
	0.1	19.80	11.9	7.26
	0.25	30.40		
	0.4	40.50		
50 [a]	0	5.71		
	0.1	7.97	5.20	2.79
	0.25	10.79		
	0.4	17.20		
43 [a]	0	4.25		
	0.1	6.34	4.50	1.44
	0.25	7.83		
	0.4	10.29		
37 [b]	0	2.07	2.07	0.756
	0.1	2.83		
25 [b]	0	0.974	0.974	0.314
	0.1	1.29		
4 [b]	0	0.0696	0.0696	0.0146
	0.1	0.0841		

[a] Experimental data.
[b] Obtained by extrapolation of Arrhenius plots.

Effect of temperature

The degradation of **1** was studied at four temperatures (43–70 °C) at pH 7 and in the presence and absence of 0.4 M NaCl. The values for k_{obs}, k_0 and k_1 from the elevated temperature study are listed in Table 1. The values obtained for k_{obs} (in the presence of 0 and 0.4 M Cl^-), k_0 and k_1

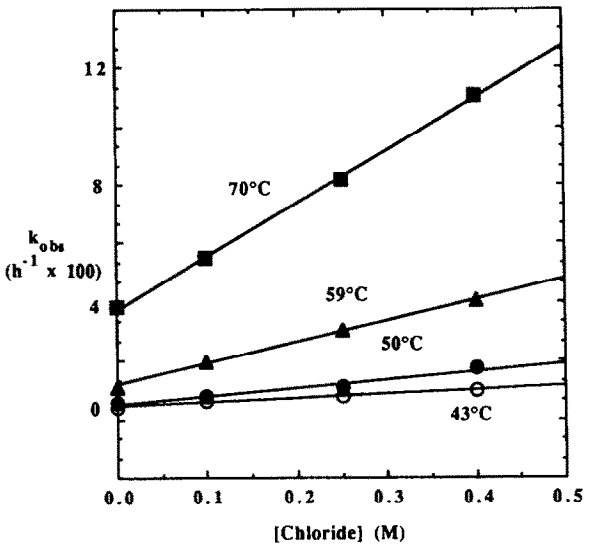


Fig. 2. Second-order plots describing the effect of chloride on the degradation of **1** at various temperatures.

at pharmaceutically relevant temperatures (4, 25 and 37 °C) were calculated from the appropriate Arrhenius plots and are also presented in Table 1. The Eyring plots for the rate constants of Eqn 1, at 70 °C, are shown in Fig. 3 and the calculated enthalpies and entropies of activation were as follows: $\Delta H^{\neq}(k_0) = 21.1 \pm 2.6$ kcal mol^{-1} (88.3 $\pm$ 11.1 kJ mol^{-1}), $\Delta S^{\neq}(k_0) - 20.0 \pm 1.0$ cal mol^{-1} K^{-1} (83.7 $\pm$ 4.4 J mol^{-1} K^{-1}), $\Delta H^{\neq}(k_1) = 19.8 \pm 1.3$ kcal mol^{-1} (82.8 $\pm$ 5.3 kJ mol^{-1}), $\Delta S^{\neq}(k_1) - 20.9 \pm 0.6$ cal mol^{-1} K^{-1} (87.4 $\pm$ 2.4 J mol^{-1} K^{-1}).

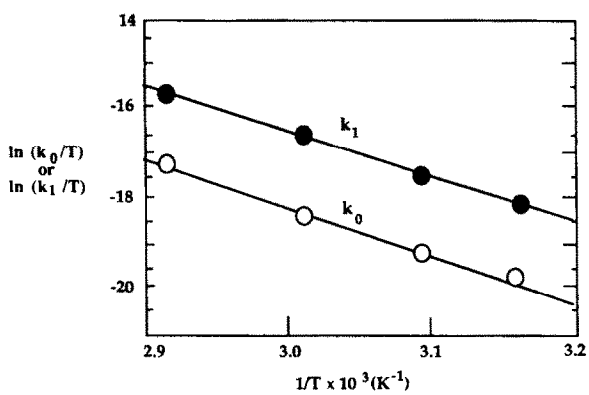


Fig. 3. Eyring plots for the rate constants k_0 and k_1 at pH 7.0 ($\mu = 1.0$).

Effect of pH

The complete log k-pH profiles for the degradation of **1** at 70 °C, in the presence and absence of 0.4 M NaCl are given in Fig. 4. Fig. 4 also shows the relationship between log k_1 and pH, the data being obtained from $k_1 = (k_{obs} - k_0)/0.4$ at each pH value. Although the effect of ionic strength on the rate was determined to be negligible at pH 7.0 (data not shown), it was maintained at 1.0 because its influence could not be precluded at other pH values. The points in Fig. 4 are experimental and the lines are theoretical being derived from Eqns 2 and 3, which describe the effects of pH on the hydrolysis and the chloride substitution reactions, respectively.

$$k_0 = k_{01}a_H + k_{02} + \frac{\left(k_{03}\frac{K_w}{k_a}\right)K_a}{K_a + a_H} + \frac{k_{04}K_w}{a_H} \qquad (2)$$

$$k_1 = \frac{(k_{11}a_H)a_H}{K_a + a_H} + \frac{(k_{12}a_H)K_a}{K_a + a_H} \qquad (3)$$

Substituting Eqns 2 and 3 into Eqn 1 gives the overall phenomenological expression (Eqn 4) describing the influences of both hydrogen ion activ-

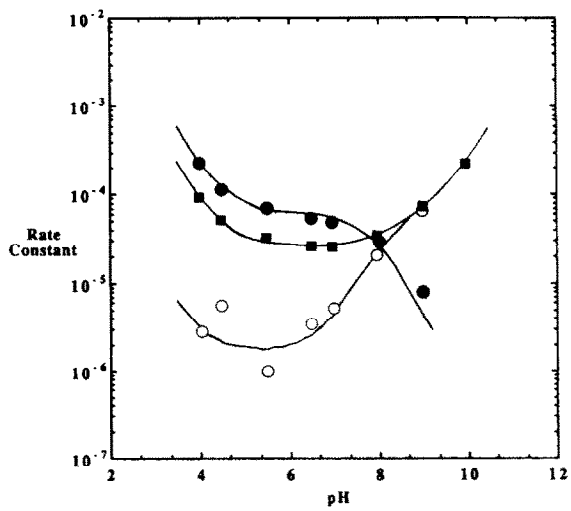


Fig. 4. Relationships between k_0, k_1 and k_{obs}([Cl$^-$] = 0.4 M) and pH for the degradation of **1** in aqueous solution at 70 °C. The points are experimental and the lines were fitted by Eqn 2 for k_0(○), 3 for k_1 (●) and 4 for k_{obs} (■).

ity and chloride ion concentration on the degradation of **1**:

$$k_{obs} = k_0 = k_{01}a_H + k_{02} + \frac{\left(k_{03}\frac{K_w}{K_a}\right)K_a}{K_a + a_H} + \frac{k_{04}K_w}{a_H} + \left\{\frac{(k_{11}a_H)a_H}{K_a + a_H} + \frac{(k_{12}a_H)K_a}{K_a + a_H}\right\}[Cl^-] \quad (4)$$

The rate constants k_{01}, k_{02}, k_{03}, k_{04}, k_{11} and k_{12}, as well as the apparent dissociation constant K_a (Table 2), may, of course, be a complex combination of rate constants.

Formulation of the rate expression

Possible mechanisms Mechanisms for the loss of **1**, consistent with these findings are summarized in Scheme 2, which postulates that **1** reacts with water, chloride or hydroxide ion to give a pentavalent intermediate (Belluco et al., 1965), in low steady-state concentrations, which then decomposes by three similar pathways to either **2** or **3** (Scheme 1).

In Scheme 2, k^w_{1,SH_2}, k^C_{1,SH_2} and k^b_{1,SH_2} are the rate constants for nucleophilic substitution of the fully protonated SH_2^+ by water, chloride and hydroxide ion, respectively, to give the pentavalent intermediate and k^w_{2,SH_2}, k^C_{2,SH_2} and k^b_{2,SH_2} are the corresponding rate constants for the reverse processes. The rate constants k^w_{3,SH_2}, k^C_{3,SH_2} and k^b_{3,SH_2} describe the cleavage of the Pt-O bond and conversion of the intermediate to either the aquo or chloro complexes (**2**, **3** and **4**) (Scheme 1). It is further postulated that **1** can exist in three ionic forms (SH_2^+, SH, and S^-) and Scheme 2 also shows the corresponding reactions for SH and S^-.

TABLE 2

Microscopic rate constants for the hydrolysis (Eqn 2) and the chloride substitution (Eqn 3) of **1** *at 70°C (μ = 1.0)*

Constant	Value
k_{11}	$1.66\ M^{-1}\ s^{-1}$
k_{12}	$4.97\times10^{3}\ M^{-1}\ s^{-1}$
$K_a(pK_a)$ [a]	1.12×10^{-8} (7.95)
$K_a(pK_a)$ [b]	4.76×10^{-9} (8.32)
k_{01}	$1.65\times10^{-2}\ M^{-1}\ s^{-1}$
k_{02}	$1.91\times10^{-6}\ s^{-1}$
k_{03}	$1.75\ M^{-1}\ s^{-1}$
k_{04}	$9.34\times10^{-2}\ M^{-1}\ s^{-1}$

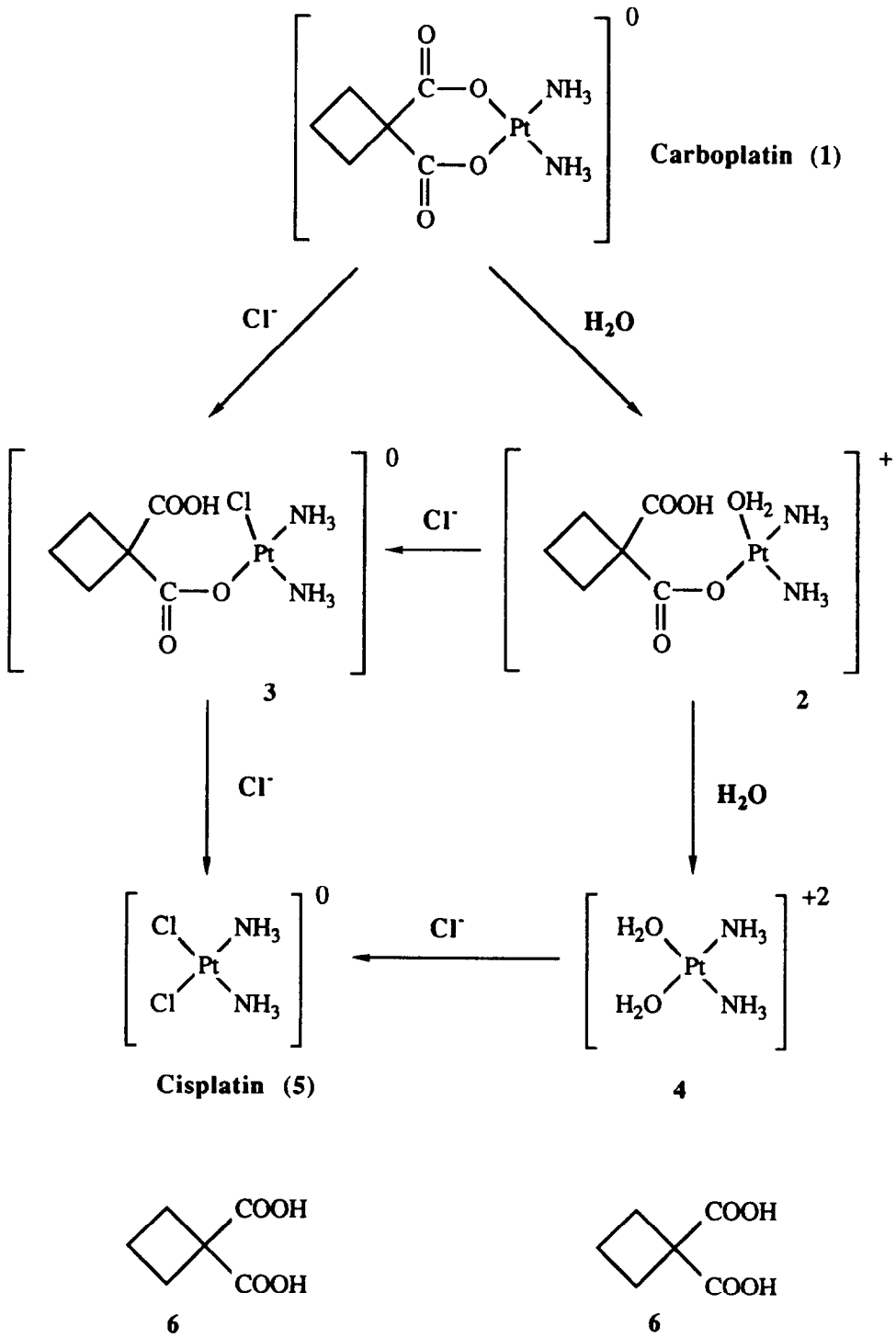
[a] Calculated from Eqn 2.
[b] Calculated from Eqn 3.

Complete mechanistic rate law According to Scheme 2a–c, the steady state kinetic law for these mechanisms is:

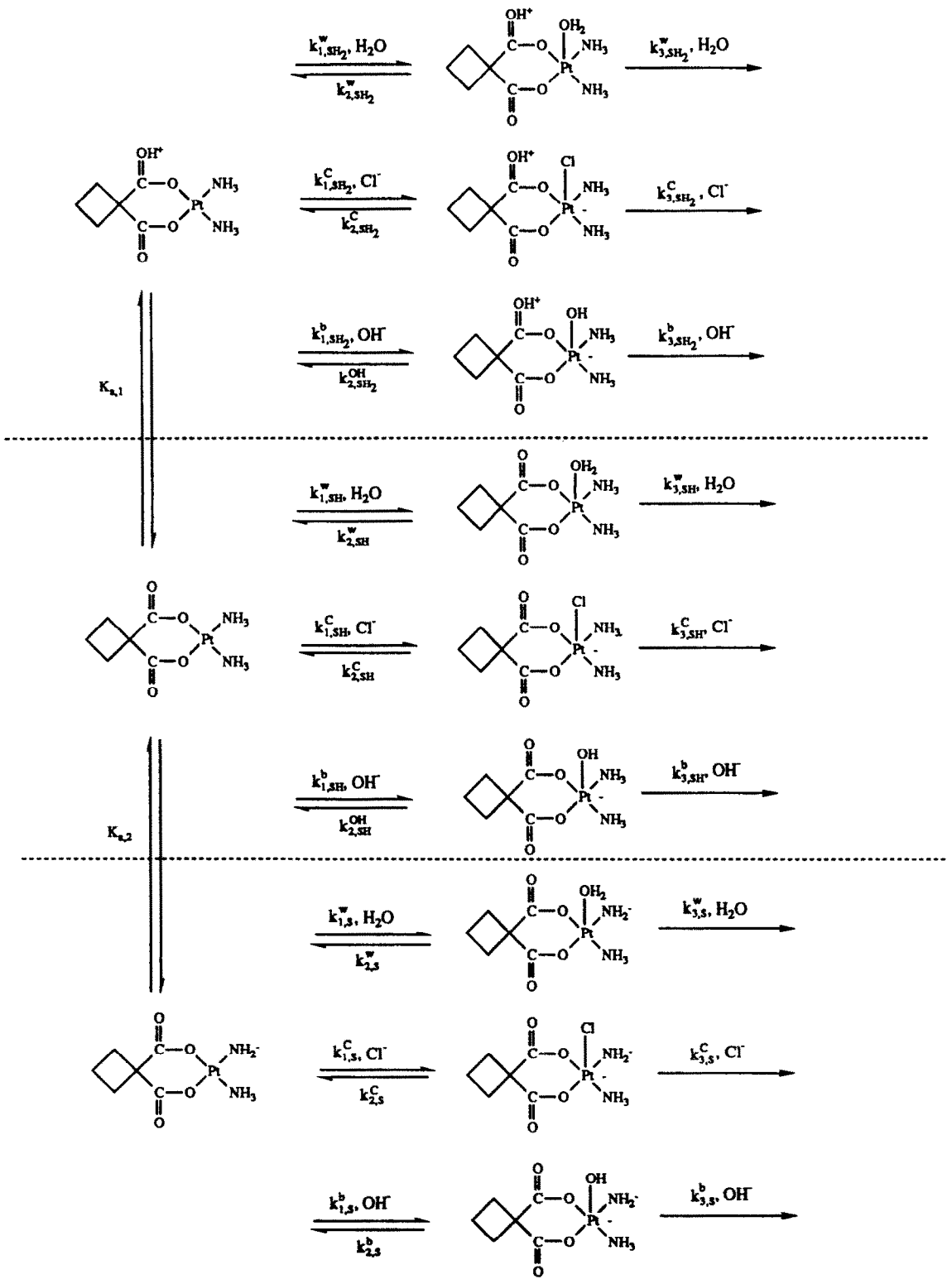
$$k_{obs} = \frac{1}{K_{a,1}K_{a,2} + K_{a,1}a_H + a_H^2}a_H^2 \times \left\{\left[\frac{k^w_{1,SH_2}k^w_{3,SH_2}}{k^w_{2,SH_2} + k^w_{3,SH_2}} + \frac{k^C_{1,SH_2}k^C_{3,SH_2}}{k^C_{2,}k_{SH_2} + k^C_{3,SH_2}}[Cl^-] + \frac{k^b_{1,SH_2}k^b_{3,SH_2}}{k^b_{2,SH_2} + k^b_{3,SH_2}}\frac{K_w}{a_H}\right] + K_{a,1}a_H\left[\frac{k^w_{1,SH}k^w_{3,SH}}{k^w_{2,SH} + k^w_{3,SH}} + \frac{k^C_{1,SH}k^C_{3,SH}}{k^C_{2,SH} + k^C_{3,SH}}[Cl^-] + \frac{k^b_{1,SH}k^b_{3,SH}}{k^b_{2,SH} + k^b_{3,SH}}\frac{K_w}{a_H}\right] + K_{a,1}K_{a,2}\left[\frac{k^w_{1,S}k^w_{3,S}}{k^w_{2,S} + k^w_{3,S}} + \frac{k^C_{1,S}k^C_{3,S}}{k^C_{2,S} + k^C_{3,S}}[Cl^-] + \frac{k^b_{1,S}k^b_{3,S}}{k^b_{2,S} + k^b_{3,S}}\frac{K_w}{a_H}\right]\right\} \quad (5)$$

Eqn 5 can be simplified to give Eqn 7 by defining $k^w_{SH_2}$ as:

$$k^w_{SH_2} = \frac{k^w_{1,SH_2}k^w_{3,SH_2}}{k^w_{2,SH_2} + k^w_{3,SH_2}} \quad (6)$$



Scheme 1. Overall reaction of **1** with chloride ion in aqueous solution.



Scheme 2. Proposed mechanism for the degradation and dissociation of 1 in aqueous solution containing chloride.

and so on.

$$k_{obs} = \frac{1}{K_{a,1}K_{a,2} + K_{a,1}a_H + a_H^2} a_H^2 \times \left\{ \left[k^w_{SH_2} + k^C_{SH_2}[Cl^-] + k^b_{SH_2}\frac{K_w}{a_H} \right] + K_{a,1}a_H \left[k^w_{SH} + k^C_{SH}[Cl^-] + k^b_{SH}\frac{K_w}{a_H} \right] + K_{a,1}K_{a,2} \left[k^w_S + k^C_S[Cl^-] + k^b_S\frac{K_w}{a_H} \right] \right\} \quad (7)$$

From Eqn 7, the separate mechanistic kinetic rate laws describing the hydrolysis (Eqn 8) and chloride substitution (Eqn 9) of **1** are given as follows:

$$k_0 = \left(a_H^2 k^w_{SH_2} + a_H \left[K_{a,1}k^w_{SH} + k^b_{SH_2}K_w \right] + \left[K_{a,1}k^b_{SH}K_w + K_{a,1}K_{a,2}k^w_S \right] + K_{a,1}K_{a,2}k^b_S\frac{K_w}{a_H} \right) \times \left(K_{a,1}K_{a,2} + K_{a,1}a_H + a_H^2 \right)^{-1} \quad (8)$$

$$k_1 = \frac{a_H^2 k^C_{SH_2} + K_{a,1}a_H k^C_{SH} + K_{a,1}K_{a,2}k^C_S}{K_{a,1}K_{a,2} + K_{a,1}a_H + a_H^2} \quad (9)$$

Hydrolysis reaction Assuming that a_H^2 is, in the pH range studied, much less than the sum of the other two terms in the denominator of Eqn 8, then Eqn 8 can be rewritten as Eqn 10, which can be reduced to the same form as the phenomenological Eqn 2:

$$k_0 = \frac{a_H^2 k^w_{SH_2}}{K_{a,1}K_{a,2} + K_{a,1}a_H} + \frac{+a_H\left[K_{a,1}k^w_{SH} + k^b_{SH_2}K_w \right]}{K_{a,1}K_{a,2} + K_{a,1}a_H} + \frac{\left[k^b_{SH}K_w + K_{a,2}k^w_S \right]}{K_{a,2} + a_H} + \frac{K_{a,2}k^b_S\frac{K_w}{a_H}}{K_{a,2} + a_H} \quad (10)$$

Each of the four terms in the numerator of Eqn 10 is important only in a particular pH region: the first term, in a_H^2, contributes only below pH 5; the second term, in a_H^1, contributes only at pH 5–7; the third term, in a_H^0, contributes only at pH 7–8.5 and the fourth term, in a_H^{-1}, only contributes above pH 8.5. The denominator of Eqn 10 is approximated below pH 7 by only the term in a_H, while both terms contribute above pH 7. Thus:

$$k_0 = \frac{k^w_{SH_2}}{K_{a,1}}a_H + k^w_{SH} + \frac{k^b_{SH_2}K_w}{K_{a,1}} + \frac{\left[k^b_{SH}K_w + K^w_S K_{a,2} \right]}{K_{a,2} + a_H} + \frac{K_{a,2}k^b_S\frac{K_w}{a_H}}{K_{a,2} + a_H} \quad (11)$$

and the terms in Eqn 11 are related to the terms in Eqn 2 as follows:

$$k_{01} = \frac{k^w_{SH_1}}{K_{a,1}} \quad (12)$$

$$k_{02} = \left(k^w_{SH} + k^b_{SH_2}\frac{K_w}{K_{a,1}} \right) \quad (13)$$

$$k_{03} = \left(k^b_{SH}\frac{K_w}{K_{a,2}} \right) + k^w_S \quad (14)$$

$$k_{04} = k^b_S \quad (15)$$

Nucleophilic substitution by chloride In a similar fashion, Eqn 9 describing the chloride-dependent reactions can be rewritten as Eqn 16, which has the same form as the phenomenological Eqn 3:

$$k_1 = \frac{a_H^2 k^c_{SH_2}}{K_{a,1}K_{a,2} + K_{a,1}a_H} + \frac{a_H k^C_{SH} + K_{a,2}k^C_S}{K_{a,2} + a_H} \quad (16)$$

The first term in the right-hand side of Eqn 19 is dominant between pH 4 and 8 and at higher pH values the second term dominates. Because Eqn 16 is of the same form as Eqn 3, the empirical rate constants in Eqn 3 may be related to the proposed

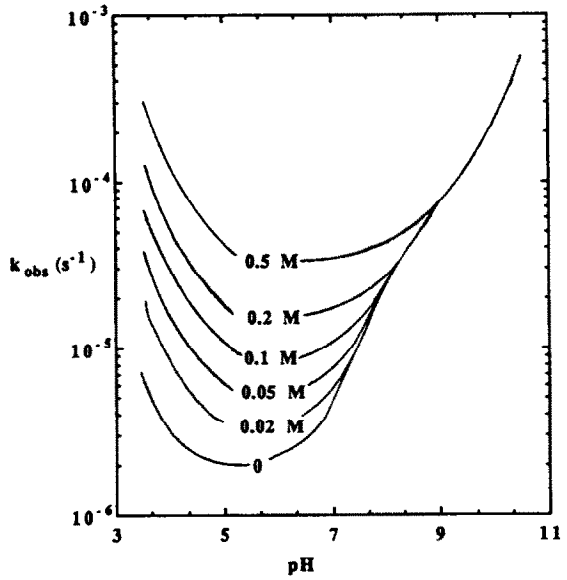


Fig. 5. Theoretical log k_{obs}-pH profiles for **1** (Eqn 4) in the presence of various concentrations of chloride ($\mu = 1.0$).

mechanistic rate constants by Eqns 17 and 18:

$$k_{11} = \frac{k^{C}_{SH_2}}{K_{a,1}} \tag{17}$$

$$k_{12} = \frac{k^{C}_{SH} a_H}{K_{a,2}} \tag{18}$$

Eqn 16 predicts that k_1 should approach a limiting value equal to k^{C}_{S} at higher values of pH; however, this limiting value was not observed because the hydroxide ion catalyzed contribution (k_{12}) to the overall rate dominates the reaction at higher pH values. Fig. 5, which shows theoretical curves calculated from Eqn 4 using the constants in Table 2, emphasizes the dominating role played by the hydroxide ion catalyzed reaction such that the contribution of added chloride ion (up to 0.5 M) to the overall rate of degradation of **1** is negligible above pH 9.

Linear free energy relationships

Linear free energy relationships (LFERs) have been used extensively to describe the nucleophilic substitution of square planar complexes of platinum(II). In this regard, the most useful nucleophilicity index is that described by Belluco et al. (1965) who defined the n^{0}_{Pt} scale (Eqn 19) by measuring the reactivity (Eqn 1) of several platinum(II) complexes in methanol. The most widely used n^{0}_{Pt} scale is based on the reactivity of *trans*-$[Pt(py)_2Cl_2]$ in methanol at 30 °C:

$$n^{0}_{Pt} = \log\left(\frac{k_1}{k_0}\right)_0 \tag{19}$$

Fig. 6 shows a non-linear relationship between the log k_1 values obtained for **1** in this study and the n^{0}_{Pt} values described by Belluco et al. (1965), which is in sharp contrast with the linear relationship described previously by Riley et al. (1982) for the reactivity of **5** with the same nucleophiles.

With nucleophiles of $n^{0}_{Pt} \sim 2$, **5** and **1** are equally reactive. But below this value, **1** appears considerably more sensitive to nucleophilicity while above this value **5** appears to be more sensitive to nucleophilicity. Thus, **1** is several orders of magnitude less reactive than **5** with water. These results suggest that mechanism for the nucleophilic substitution of **1** proceeds in a manner similar to that for **5** when the nucleophile is relatively strong, but that there is change in the rate determining step, in the case of **1**, when the nucleophile is relatively weak.

TABLE 3

Second order rate constants, k_1 (Eqn 1) for the reaction of **1** *with various nucleophiles and their corresponding nucleophilicity values*

Nucleophile	k_1 [a] ($M^{-1}\,h^{-1}$)	Nucleophilicity index		
		E^{0} [b]	$pk_{CH_3Hg^+}$ [b]	n^{0}_{Pt} [c]
H_2O	1.93×10^{-5}	−2.60	–	0
Cl^-	3.20×10^{-3}	−1.36	5.45	1.65
OH^-	–	−0.95	9.50	≤1
N_3^-	0.18	−1.02	–	2.19
I^-	1.13	−0.54	8.70	4.03
SCN^-	1.20	−0.77	6.10	4.26
$S_2O_3^{2-}$	8.14	+0.30	10.95	5.95

[a] Eqn 1.
[b] Describes reactivity at carbon (Belluco et al., 1965).
[c] Describes reactivity at platinum (Belluco et al., 1965).

Mechanistic analysis

Hydrolysis For the region of pH 4–5, the rate constant for hydrolysis, k_0 is given by:

$$k_0 = \left(\frac{k^{w}_{1,SH_2} k^{w}_{3,SH_2}}{k^{w}_{2,sH_2} + k^{w}_{3,SH_2}} \right) \frac{K_{a,1}}{a_H} \qquad (20)$$

which corresponds to the mechanism shown in Scheme 3.

In the pH-independent region (pH 5–7), k_0 is dominated by the two kinetically equivalent reactions which can be described by Eqn 21 and by the reactions shown in Scheme 4. The first mechanism involving attack by water on **1** is preferred to the second mechanism involving attack by hydroxde ion on the protonated form of **1** because the reaction was shown to be independent of ionic strength at pH 7.

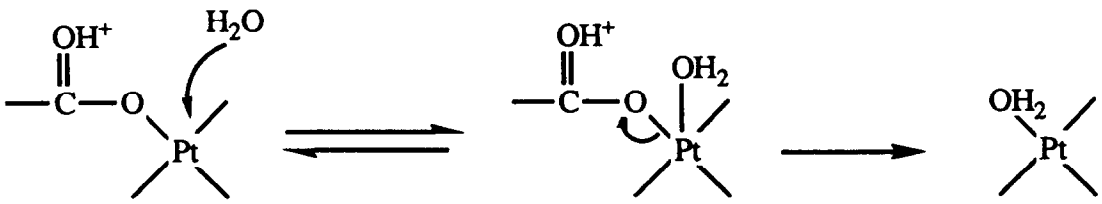
$$k_0 = \left(\frac{k^{w}_{1,SH} k^{w}_{3,SH}}{k^{w}_{2,SH} + k^{w}_{3,SH}} \right) + \left(\frac{k^{b}_{1,SH_2} k^{b}_{3,SH_2}}{k^{b}_{2,SH_2} + k^{b}_{3,SH_2}} \right) \left(\frac{K_w}{K_{a,1}} \right) \qquad (21)$$

In the alkaline region (pH 7–10), the hydrolysis of **1** is dominated by base catalysis and is modulated by the presence of an ionizable functional group ($-NH_3 \rightarrow NH_2^-$). The overall mechanistic rate law for this pH region is given by Eqn 22:

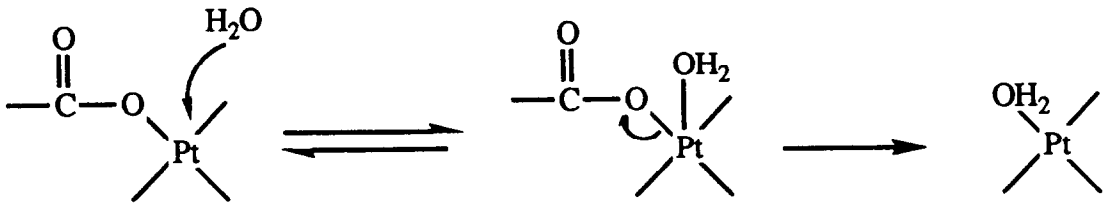
$$k_0 = \left\{ \left(\frac{k^{b}_{1,SH} k^{b}_{3,SH} K_w}{k^{b}_{2,SH} + k^{b}_{3,SH}} \right) + \left(\frac{k^{w}_{1,S} k^{w}_{3,S} K_{a,2}}{k^{w}_{2,S} + k^{w}_{3,S}} \right) + \left(\frac{k^{w}_{1,S} k^{w}_{3,S} \frac{K_{a,2}}{a_H}}{k^{w}_{2,S} + k^{w}_{3,S}} \right) \right\} \frac{1}{K_{a,2} + a_H} \qquad (22)$$

The mechanism proposed in Scheme 2 involves initial nucleophilic attack by the hydroxide ion at the platinum atom to form the steady state, pentavalent intermediate. However, the hydroxide is known to be a very poor nucleophile for platinum complexes and has been assigned an n^0_{Pt} value less than 1 by Belluco et al. (1965), which leads one to question the mechanism for the hydroxide ion catalyzed reaction proposed in Scheme 2. Furthermore, Table 3 shows that the reactivities of the hydroxide ion ($k_{03} = 1.75\ M^{-1}\ h^{-1}$) and the chloride ($k_{11} = 1.66\ M^{-1}\ h^{-1}$) with respect to **1** are similar at pH 7.0 where the predominant species is SH. This is also at odds with their n^0_{Pt} values. The relative reactivities of Cl^- and OH^- are also at odds with what would be predicted from their standard electrode potentials (Table 3). **1** will exist in its S^- form, at higher pH values ($pK_{a,2} = 7.95$) and the presence of the negative charge on the complex leads to substantial reduction in the hydroxide ion catalytic rate constant ($9.34 \times 10^{-2} M^{-1}\ h^{-1}$).

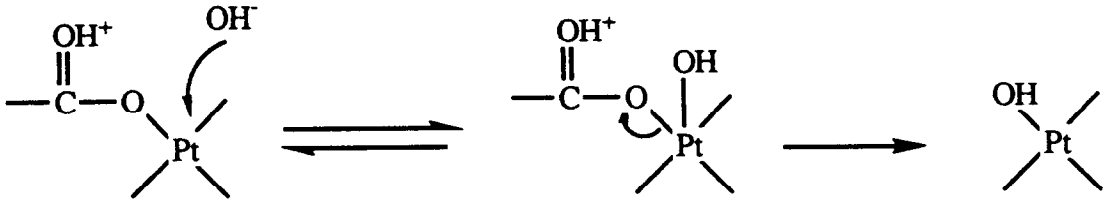
These observations lead to the postulation of an alternative mechanism (Scheme 5; cf. Scheme 2) for the base-catalyzed hydrolysis of **1** in which the reaction proceeds via hydroxide-ion attack at the carbonyl-carbon atom. Table 3 shows that the reactivity of OH^- for nucleophilic substitution at carbon is much higher than that of Cl^-. This suggests that the similarity in the rate constants for the reaction of Cl^- and OH^- with **1** is coincidental and that they reflect the reactivities of the two nucleophiles at different centers in **1**. Unfortunately, the actual mechanism cannot be deduced from the kinetics alone, because the kinetic expression in Eqn 22 is consistent with either mechanism and the reaction may proceed by a mixture of both.



Scheme 3. Proposed mechanism for the acid-catalyzed hydrolysis of **1**.



which is equivalent to:

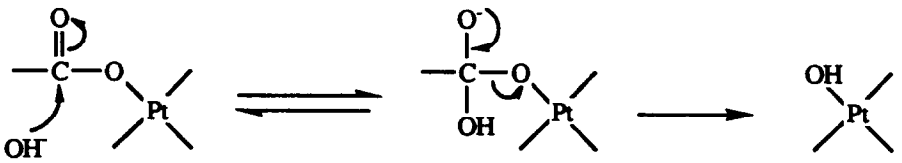


Scheme 4. Proposed mechanism for the neutral- (water-) catalyzed hydrolysis of **1**.

Nucleophilic substitution by chloride ion Over the pH range of 4–10, nucleophilic substitution of **1** by chloride proceeds via acid catalysis (Scheme 6) with the negatively charged S^- form of **1** being substantially more reactive than the SH form. The proposed mechanism for acid catalyzed nucleophilic substitution of **1** by chloride ion is shown in Scheme 6 and the overall equation consistent with this mechanism is given in Eqn 23 which is obtained by expansion of Eqn 16.

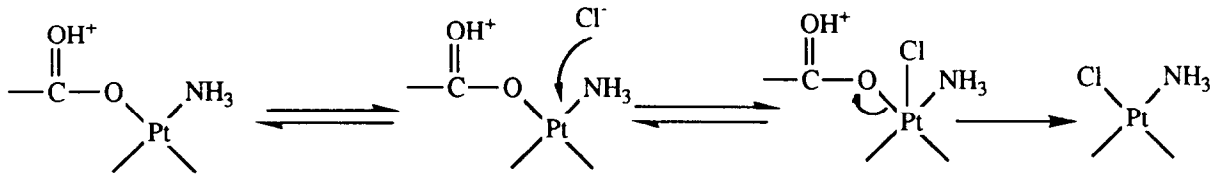
$$k_0 = \left\{\left(\frac{k^C_{1,SH_2}k^C_{3,SH_2}a_H^2}{(k^C_{2,SH_2}+k^C_{3,SH_2})(K_{a,1}K_{a,2}+K_{a,1}a_H)}\right)\right\} + \left\{\left(\frac{k^C_{1,SH}k^C_{3,SH}a_H}{(k^C_{2,SH}+k^C_{3,SH})}\right) + \left(\frac{k^C_{1,SH}k^C_{3,SH}K_{a,2}}{(k^C_{2,SH}+k^C_{3,SH})}\right)\right\}\left(\frac{1}{K_{a,2}+a_H}\right) \quad (23)$$

Non-linear free energy relationship Relationships in which attack of the nucleophile is rate-determining (k_1 steps, Schemes 4–6) are expected to have smaller values of $\partial \log k/\partial n^0_{Pt}$ than reactions with decomposition of the adduct rate-determining (k_3 steps in Schemes 4–6). This is because the transition state for attack has only a partial bond of the nucleophile to Pt and ΔG^* should not be highly sensitive to nucleophilicity, while the transition state for adduct decomposition has a full Pt-nucleophile bond and ΔG^* should therefore be maximally sensitive to nucelophilicity. The plot for **1** in Fig. 6 has the classical form for a reaction in which the rate determining step at $n^0_{Pt} \leqslant 2$ is adduct decomposition but changes to adduct formation with the most powerful nucleophiles. This was expected because the most powerful nucleophiles will be difficult to re-expel, making their binding nearly irreversible. Cisplatin appears to have a single rate-limiting step throughout. This is almost surely the addition step because the high reactivity of chloride com-

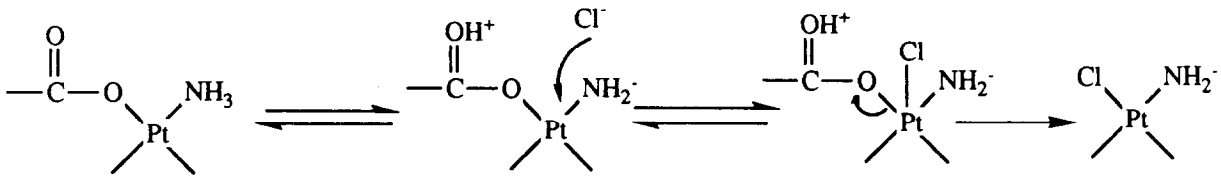


Scheme 5. Alternative mechanism (cf. Scheme 2) for the hydroxide ion catalyzed hydrolysis of **1**.

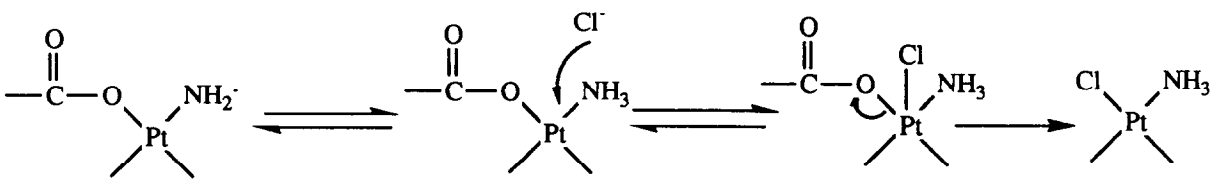
a) pH region 4 - 7



b) pH region 7 - 10



which is equivalent to:



Scheme 6. Proposed mechanisms for the acid catalyzed nucleophilic substitution of **1** by chloride ion.

pared with carboxylate will make adduct decomposition much faster with **5** than with **1**.

Pharmaceutical and biopharmaceutical implications

The reactions of **1** with water and chloride ions are of both pharmaceutical and clinical significance. At 25 °C the times for 5% degradation ($t_{0.95}$) of **1** in water and in 0.9% sodium chloride calculated from the rate constants presented in Table 2, were 52.7 and 29.2 h, respectively. This is consistent with previous work (Sewell et al., 1987b), which showed 3% loss of **1** in the absence of chloride ions after 24 h at ambient temperature (22–23 °C). **1** should not be reconstituted with 0.9% sodium chloride solution for prolonged continuous infusion regimes which may be administered over 5–30 days (Sewell et al., 1987b). These rate constant data enable the prediction of the stability of **1** in admixtures of chemotherapeutic agents where chloride may be present as a formulation excipient or as a drug counterion.

1 can undergo similar reactions in plasma where the chloride concentration is approx. 0.1 M. The calculated rate constant (k_{obs}) and corresponding half-life for degradation in the presence of 0.1 M Cl^- at 37 °C are shown in Table 2. The calculated half-life of 244 h considerably exceeds the reported half-lives for **1** in plasma ultrafiltrate of 29 h (Harland et al., 1984) and 32 h (Gaver and Deeb, 1986) obtained in vitro. Since **1** is not

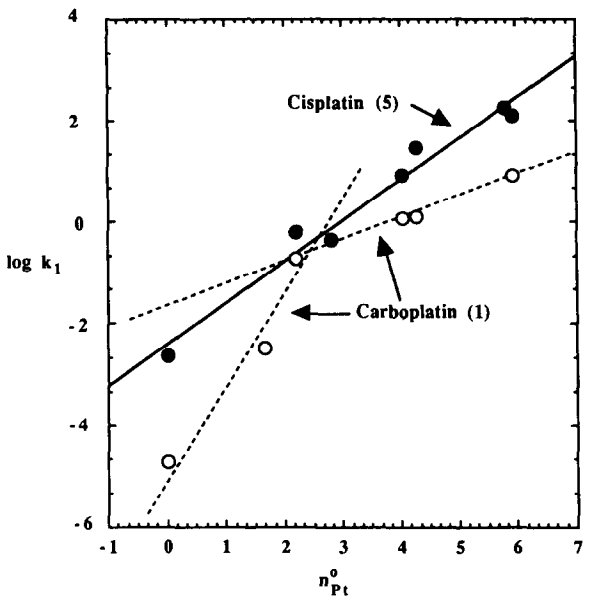


Fig. 6. Linear free energy relationships for the reactions of cisplatin (5) and 1 with various nucleophiles. The data for 5 (at 30 °C) have been taken from Riley et al. (1982) and the data for 1 (at 25 °C) are from this study.

highly protein bound in plasma (Oguri et al., 1988), the rapid loss of carboplatin in vitro may be explained by its reaction with other nucleophiles in plasma.

This study has also shown that the degradation of 1 is accelerated in the presence of a variety of nucleophiles. The nucleophiles chloride, iodide and thiocyanate are present in the blood and may effect the non-enzymic degradation of carboplatin in vivo. When these nucleophiles react with 1, different species of the drug are formed which may contribute to the toxicity, protein binding and clinical activity of 1. The formation of ionic species from the hydrolysis of 1 or its nucleophilic substituted intermediates could adversely affect the passive diffusion of platinum across cell membranes.

Finally, the present study has demonstrated that the reactivity of 1 with strong nucleophiles is very similar to that of cisplatin (5), the lead compound in this series of antineoplastic drugs. In contrast, 1 is much less reactive than 5 with respect to weak nucleophiles such as water and chloride. The difference in nucleophilic selectivity of 1 compared with 5 described here (Fig. 6) is an important result of potential clinical significance, because it may be related to the differences in the toxicities of these two drugs and is worthy of further investigation. In particular, further studies are in progress to isolate platinum-containing complexes arising from the non-enzymic degradation of 1 in vivo, and to evaluate the significance of these complexes with toxicity and radiosensitization studies in mammalian cell culture systems.

Conclusions

The degradation of carboplatin obeyed pseudo first-order kinetics. In the presence of added nucleophiles, the observed pseudo first-order rate constant (k_{obs}), comprised two components, i.e.: $k_{obs} = k_0 + k_1[Nu]$, where k_0 and k_1 are the hydrolytic and nucleophile dependent rate constants respectively. The reaction with water (k_0) proceeded with acid catalysis (pH 4–5), by uncatalyzed routes (pH 5–7) and by base catalysis (pH 7–10). On the other hand, nucleophilic attack by chloride proceeded by acid catalysis only. The data were consistent with a mechanism involving attack by water or other nucleophiles to form a pentavalent platinum complex, at low steady-state concentrations, which then degraded by cleavage of a Pt-O bond. The higher than expected reactivity of carboplatin with the hydroxide ion suggested that the hydrolysis of carboplatin in basic solutions proceeds via an alternative mechanism involving attack by the hydroxide ion at the carbonyl carbon atom.

The data were used to predict carboplatin degradation rates at temperatures of pharmaceutical and clinical significance. At 25 °C the $t_{0.95}$ values for carboplatin were 52.7 h in water and 29.2 h in 0.9% sodium chloride. Therefore, carboplatin should not be reconstituted with 0.9% sodium chloride for use in continuous infusion regimes where long term drug stability (< 5% loss) is essential. In plasma the chloride ion concentration is approx. 0.1 M. At 37 °C in aqueous solution containing 0.1 M Cl^- the carboplatin half-life was estimated to be 244 h which contrasts with the reported plasma half-life of 29–32 h obtained in

vitro. This difference was attributed to the reaction of carboplatin with other plasma nucleophiles and possibly to protein binding of carboplatin.

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