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Computational consideration of cisplatin hydrolysis and acid dissociation in aqueous media: effect of total drug concentrations

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Abstract

Cisplatin (*cis*-DDP) is subject to nucleophilic displacement of chloride in water, forming aquated species, subsequently liberating hydrogen ion(s) with increasing pH. This study intends to theoretically analyze the hydrolysis and polyprotic dissociation behavior of *cis*-DDP in various aqueous media. A mathematical model was expressed by nonlinear simultaneous equations in terms of the total drug concentration, pH and pCl based on the hydrolysis and acid dissociation constants already published. Some of the interesting simulation results include that (1) in water, *cis*-DDP behaves in a very complicated manner, highly depending on the total drug concentration, pH and pCl, (2) in normal saline, about 3% of the total concentration is a positively charged chloro-aqua that may be very reactive, (3) in assumed blood (pH 7.4, $[Cl^-] = 0.11$ mol/l, $\mu = 0.15$), the drug is stabilized at the level of 85% and the remnants are the chloro-hydroxo (11%) and the chloro-aqua (4%), (4) in assumed intracellular conditions (pH 7.1, $[Cl^-] = 0.01$ mol/l, $\mu = 0.15$), the drug is converted to a large extent to various species including the parent species (44%), the chloro-hydroxo (30%), hydroxo-aqua (2%), chloro-aqua (24%) diaqua (less than 1%) and dihydroxo (null). The results of this analysis may provide a useful preliminary knowledge of existing species in a system concerned and a rationale for re-evaluating the reactions between *cis*-DDP and various nucleophilic substances already reported while there are somewhat conflicting interpretations of some *cis*-DDP reactions.

Keywords: Cisplatin; Dissociation in aqueous media; Mathematical model; Simulation; Generalized Newton method

1. Introduction

Cisplatin (*cis*-DDP) has been widely used in the treatment of solid malignant cancers such as testicular, bladder and ovarian tumors (Lyss, 1992). The drug is a square coordination platinum compound with two amine moieties and two chlorides in the *cis*-orientation. Its chemical characteristics in aqueous media have been extensively studied in view of the hydrolysis and acid dissociation behavior and chemical species responsible for biological activities (Reishus and Martin, 1961;

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Perumareddi and Adamson, 1968; Johnson et al., 1980; Bose et al., 1986; Boreham et al., 1981; Appleton et al., 1989; Miller and House, 1989a,b, 1990). The *cis*-DDP hydrolysis was first designated by Jensen (1936) with regard to the increase in conductivity of the solution with time as follows:

$$cis-\mathrm{Pt(NH_3)_2Cl_2}+\mathrm{H_2O} \rightleftarrows cis\mathrm{Pt(NH_3)_2Cl(OH_2)^+}+\mathrm{Cl^-}$$

$$cis-\mathrm{Pt(NH_3)_2Cl(OH_2)^+}+\mathrm{H_2O} \rightleftarrows cis\mathrm{Pt(NH_3)_2(OH_2)_2^{2+}}+\mathrm{Cl^-}$$

Those reactions, however, are restricted only to acidic conditions. The resulting aquated species are further dissociated to liberate hydrogen ions as the pH increases. The whole scheme of hydrolysis and polyprotic dissociation of *cis*-DDP in aqueous media is expressed in terms of its three positively charged aquated species, three electrically neutral species including the parent species and half mol of a dimer (Fig. 1).

After *cis*-DDP was frequently aged in pure water for a few days, where the full conversion into the aquated species is assumed, its reactivity was examined with nucleophilic substances such as 2-deoxy-D-glucose and 5′-guanosine monophosphate (Shearan et al., 1990; Chen et al., 1994; Zheng et al., 1997; Zenker et al., 2000). However, little attention was paid to the fractions of various

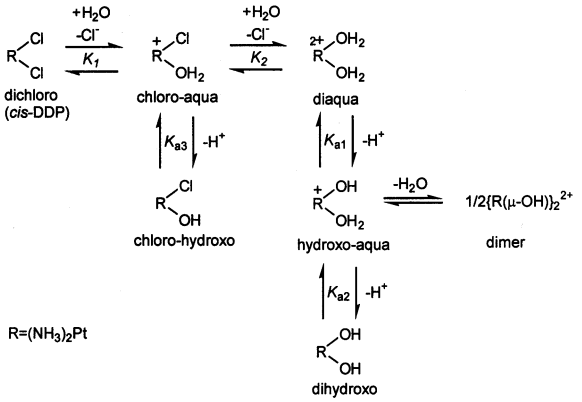

Fig. 1. Hydrolysis and acid dissociation scheme of *cis*-DDP in aqueous media.

existing species, especially reactive species, while they were considered to largely depend on the total drug concentration, pH and pCl in a fresh reaction system concerned.

The simultaneous determinations of various products produced from *cis*-DDP would almost be impossible by conventional analytical methods like an HPLC assay and acid–base titration. To overcome these problems, the computer simulation of existing species under a variety of conditions may provide useful information to pursue its reactions with other substances. It is also to provide some insight about the therapeutically active species of the drug at the deep-seated site of action in the physiological conditions where its local concentration would be extremely low.

This study intends to theoretically analyze the comprehensive dissociation behavior of *cis*-DDP at equilibria over a wide range of the total drug concentration based on the integration of hydrolysis and acid dissociation constants published elsewhere (Table 1).

2. Materials and methods

2.1. Materials

cis-DDP was purchased from Sigma (St. Louis, MO) and used without further purification. The solubility of the drug in water is 8.43E−3 mol/l (0.253 g per 100 g of water, Merck Index, 13th ed.) at 25 °C. *cis*-DDP was dissolved in distilled water that was left with air contact for 2 days before used. pHs were monitored on a Horiba F-12 pH meter equipped with a combination electrode (Model 6366-10D, Horiba, Kyoto) calibrated with standard buffer solutions at pHs 4.01 and 6.86 (Katayama Chemical Co., Tokyo)

2.2. Dissociation model for cis-DDP in aqueous media

cis-DDP is subject to hydrolysis to form two aquated species and is further dissociated as a triprotic acid. The equilibrium constants were given for its hydrolysis and acid dissociation products (Fig. 1). It is qualitatively obvious that

Table 1
Hydrolysis and acid dissociation constants of *cis*-DDP in aqueous media compiled from literature(25 °C)

Medium	Equilibrium constants (mol/l)					Reference
	K_1	K_2	K_{a1}	K_{a2}	K_{a3}	
H_2O	3.86E−3	–	2.34E−6	5.62E−10	–	Perumareddi and Adamson (1968)
$\mu = 0.318$ (KNO_3)	3.63E−3	1.11E−4	–	–	–	Lee and Martin (1976)
$\mu = 0.318$ (Na_2SO_4)	3.3E−3	4E−5	–	–	–	Reishus and Martin (1961)
$\mu = 1.0$ ($HClO_4$)	1.01E−2	2.7E−4	–	–	–	Miller and House (1989b)
$\mu = 1.0$ ($HClO_4$)	1.11E−2[a]	–	–	–	–	Miller and House (1989a)
H_2O	–	–	1.17E−6[b]	1.35E−8[b]	1.41E−7	Appleton et al. (1989)
	–	–	2.75E−6[c]	4.78E−8[c]	–	Jensen (1939)

K_1, K_2, K_{a1} K_{a2} K_{a3} are the equilibrium constants designated in Fig. 1.
[a] 35 °C.
[b] 5 °C.
[c] 20 °C.

the equilibrium positions are highly affected by the total drug concentration as well as pH and pCl.

From this scheme where R designates an $(NH_3)_3Pt$ moiety, the equilibrium expressions are:

$$K_1 = \frac{[RCl(OH_2)^+][Cl^-]}{[RCl_2]} \quad (1)$$

$$K_2 = \frac{[R(OH_2)_2^{2+}][Cl^-]}{[RCl(OH_2)^+]} \quad (2)$$

$$K_{a1} = \frac{[R(OH_2)(OH)^+][H^+]}{[R(OH_2)_2^{2+}]} \quad (3)$$

$$K_{a2} = \frac{[R(OH_2)][H^+]}{[R(OH_2)(OH)^+]} \quad (4)$$

$$K_{a3} = \frac{[RCl(OH)][H^+]}{[RCl(OH_2)^+]} \quad (5)$$

The total concentration of the drug, C_t, is represented as follows:

$$C_t = [RCl_2] + [RCl(OH_2)^+] + [R(OH_2)_2^{2+}] + [R(OH_2)(OH)^+] + RCl(OH)] + [R(OH)_2] \quad (6)$$

From Eqs. (1)–(6), the concentrations of respective species may be expressed in terms of the parent species:

$$[RCl(OH_2)^+] = \frac{K_1[RCl_2]}{[Cl^-]} \quad (7)$$

$$[R(OH_2)_2^{2+}] = \frac{K_1K_2[RCl_2]}{[Cl^-]^2} \quad (8)$$

$$[R(OH_2)(OH)^+] = \frac{K_1K_2K_{a1}[RCl_2]}{[H^+][Cl^-]^2} \quad (9)$$

$$[RCl(OH)] = \frac{K_1K_{a3}[RCl_2]}{[H^+][Cl^-])} \quad (10)$$

$$[R(OH)_2] = \frac{K_1K_2K_{a1}K_{a2}[RCl_2]}{([H^+]^2[Cl^-]^2)} \quad (11)$$

where $[RCl_2]$ is given by:

$$[RCl_2] = \frac{[H^+]^2[Cl^-]^2C_t}{[H^+]^2[Cl^-]^2 + K_1[H^+]^2[Cl^-] + K_1K_2[H^+]^2 + K_1K_2K_{a1}[H^+] + K_1K_2K_{a1}K_{a2} + K_1K_{a3}[H^+][Cl^-]} \quad (12)$$

The total $[H^+]$ and $[Cl^-]$ in solution may be expressed by:

$$[H^+] = [R(OH_2)(OH)^+] + 2[R(OH)_2] + [RCl(OH)] + [OH^-] + [x]_{cd} \quad (13)$$

$$[Cl^-] = [RCl(OH_2)^+] + 2[R(OH_2)_2^{2+}] + 2[R(OH_2)(OH)^+] + [RCl(OH)] + 2[R(OH)_2] + [x]_{cl} \quad (14)$$

where $[OH^-]$ and $[x]_{cd}$ are the hydrogen ion concentrations contributed from the dissociation of water and dissolved carbon dioxide in water, respectively. The input variables include the total drug concentration, C_t the arbitrarily added chloride (ex NaCl), $[x]_{cl}$ and pH.

The mathematical model is characterized by the equations that take account of the initial pH of distilled water often dissolving carbon dioxide(pH 5.64 in this calculation). And water dissociation could not be omitted when no carbon dioxide is assumed to dissolve. These would be indisputable factors when the total drug concentration experimentally given is extremely low (ca. less than 1E−6 mol/l). The $[x]_{cd}$ could be variable between pH 5.64 and 7($[x]_{cd} = 0$) in the model.

Many workers have given the hydrolysis and acid dissociation constants of *cis*-DDP as compiled in Table 1. The values vary somewhat from one source to another.

The equilibrium constants used for calculation were as follows: in water at 25 °C, $K_1 = 3.86E-3$ ($pK_1 = 2.41$), $K_3 = 3.16E-5$($pK_2 = 4.50$), $K_{a1} = 2.34E-6$($pK_{a1} = 5.63$), $K_{a2} = 5.62E-10$($pK_{a2} = 9.25$), $K_{a3} = 1.41E-7$($pK_{a3} = 6.85$), $K_w = 1.01E-14$, $[x]_{cd} = 2.29E-6$ mol/l (pH 5.64).

In 0.9% NaCl solution ($\mu = 0.154$), the effect of ionic strength may have to be taken into account. For convenience the equilibrium constant was defined as an apparent constant, K', in terms of the hydronium or chloride ion activity, the concentration of species and the activity coefficient as follows (Martin, 1993):

$$pK' = pK + \log \frac{\gamma_{prod}}{\gamma_{react}} \quad (15)$$

where γ_{prod} and γ_{react} are the activity coefficients of product and reactant, respectively. The activity coefficient may be approximated in terms of ionic strength, μ, by the Debye–Hückel equation (Stokes and Robinson, 1948):

$$-\log \gamma_i = \frac{AZ_i^2\sqrt{\mu}}{1 + a_iB\sqrt{\mu}} \quad (16)$$

where Z is the charge on the species i. The values of A and a_iB may be taken to be approximately 0.51 and 1.0 in water, respectively, at 25 °C. The equilibrium constants used for calculation in normal saline were, therefore, as follows: $K_1 = 5.37E-3$ ($pK_1 = 2.27$), $K_2 = 8.51E-5$ ($pK_2 = 4.07$), $K_{a1} = 8.71E-7$ ($pK_{a1} = 6.06$), $K_{a2} = 4.07E-10$ ($pK_{a2} = 9.39$), $K_{a3} = 1.02E-7$ ($pK_{a3} = 6.99$), $K_w = 1.01E-14$, $[x]_{cd} = 2.29E-6$ mol/l (pH 5.64).

It was assumed that the equilibrium constants remain unchanged in terms of the total drug concentration. Also assumed was that the formation of hydroxo-bridged oligomers is negligible (Miller and House, 1990; Boreham et al., 1981).

The generalized Newton method was used for the numerical optimization to solve nonlinear simultaneous equations of $[H^+]$ and $[Cl^-]$ as a function of the total drug concentration (Stanek, 1996) (Appendix A). The Decimal BASIC (based on ISO FULL BASIC) was used for calculation (Shiraishi, 2001).

3. Results and discussion

3.1. Dissociation of cis-DDP in water

cis-DDF is usually stabilized by NaCl (0.9% w/v) in commercial products. To investigate the reactivity of the drug to various biological substances, however, the reaction is frequently aged in water without the stabilizer. As shown in Fig. 1, *cis*-DDP produces six species including their parent form. Their equilibrium fractions (or concentrations) seem to be highly affected by the background pH and pCl due to the total drug concentration.

Table 2 shows the effect of different total concentrations of *cis*-DDP (1.00E−8 to the saturated 8.43E−3 mol/l at 25 °C) on its hydrolysis

Table 2
Effect of the total *cis*-DDP concentration on the fraction changes of hydrolysis and acid dissociation species, pH and pCl changes in water[a]

Total cis-DDP (mol/l)	Hydrolysis and acid dissociation species (%)						Calculated pH (measured pH)	Calculated pCl
	R–Cl_2	R–$Cl(OH_2)^+$	R–$Cl(OH_2)_2^{2+}$	R–Cl(OH)	R–$(OH_2)(OH)^+$	R–$(OH)_2$		
1.00E−8	0.00	0.03	49.6	0.00	50.3	0.01	5.64	7.70
1.78E−8	0.00	0.06	49.7	0.00	50.3	0.01	5.64	7.45
3.16E−8	0.00	0.10	49.7	0.01	50.2	0.01	5.63	7.20
5.62E−8	0.00	0.18	49.8	0.01	50.0	0.01	5.63	6.95
1.00E−7	0.00	0.32	50.0	0.02	49.7	0.01	5.63	6.70
1.78E−7	0.00	0.56	50.2	0.03	49.2	0.01	5.62	6.45
3.16E−7	0.00	1.01	50.7	0.06	48.3	0.01	5.61	6.20
5.62E−7	0.00	1.81	51.3	0.10	46.8	0.01	5.59	5.95
1.00E−6	0.00	3.25	52.2	0.17	44.4	0.01	5.56 (5.62)	5.71
1.78E−6	0.01	5.79	53.0	0.27	40.9	0.01	5.52	5.46
3.16E−6	0.02	10.1	53.3	0.41	36.1	0.01	5.46	5.22
5.62E−6	0.04	16.9	52.2	0.59	30.3	0.00	5.39	4.99
1.00E−5	0.12	26.6	48.7	0.79	23.9	0.00	5.32 (5.34)	4.76
1.78E−5	0.28	38.4	42.6	0.96	17.7	0.00	5.25	4.55
3.16E−5	0.61	51.1	34.8	1.10	12.4	0.00	5.18	4.33
5.62E−5	1.22	62.9	26.5	1.17	8.20	0.00	5.12	4.12
1.00E−4	2.29	72.6	18.8	1.19	5.12	0.00	5.06 (5.08)	3.91
1.78E−4	4.07	79.2	12.6	1.15	3.03	0.00	5.01	3.70
3.16E−4	6.93	82.3	8.01	1.05	1.70	0.00	4.96	3.49
5.62E−4	11.3	82.0	4.88	0.92	0.91	0.00	4.90	3.27
1.00E−3	17.5	78.4	2.89	0.76	0.46	0.00	4.84 (4.71)	3.07
1.78E−3	25.4	72.1	1.67	0.60	0.23	0.00	4.77	2.87
3.16E−3	34.7	63.8	0.96	0.45	0.11	0.00	4.70	2.68
5.62E−3	44.5	54.5	0.55	0.33	0.05	0.00	4.63	2.50
8.43E−3[b]	51.4	48.0	0.37	0.26	0.03	0.00	4.58 (4.80)	2.38

R = $(NH_3)_2Pt$;
[a] Initial pH of water, 5.64, was measured and used throughout the calculation. Measured pH value: average value ($n = 3$).
[b] Saturated concentration at 25 °C.

and acid dissociation in water, where the calculated and measured pH and calculated pCl at equilibrium are also given. To make it easier to survey the whole picture, the fraction changes of respective species, pH and pCl changes were graphically shown in Fig. 2.

The calculated pH dependency on the total concentration was well in agreement with the experimentally determined values. This agreement indicates the assumptions and multiple-step calculations were confirmed valid. Several characteristics were found as a function of the total concentration: (1) the change of pH was about one unit while pCl was almost proportional to the total concentration, (2) the diaqua and hydroxo-aqua are the dominant species in the very diluted region leas than 5E−7 mol/l, the total of them reaching almost 100%, (3) little dichloro was found in the range less than 1E−4 mol/l, (4) the fraction changes seem to form a boundary across the total concentration of about 3E−7 mol/l, and (5) the diaqua and chloro-aqua showed unexpected maximal fractions at around 4E−6 and 5E−4 mol/l, respectively.

The extent of chloride displacement of *cis*-DDP is not only determined by pCl changes but also by pH. While the pH remains unchanged in the extremely diluted region of the drug less than 3E−7 mol/l, the predominant diaqua and hydroxo-aqua are due to much lower pK_1 (2.4l)

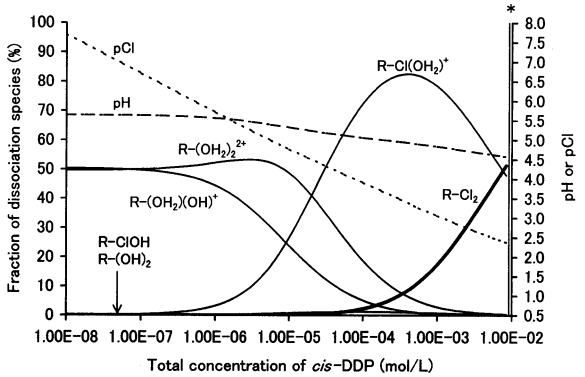

Fig. 2. Simulation of the effect of total *cis*-DDP concentration on the fraction changes of its hydrolysis and dissociation species and pH and pCl in water. R = $(NH_3)_2Pt$; *, saturated solution at 25 °C; NaCl = 0 mol/l; initial pH of distilled water assumed: 5.64.

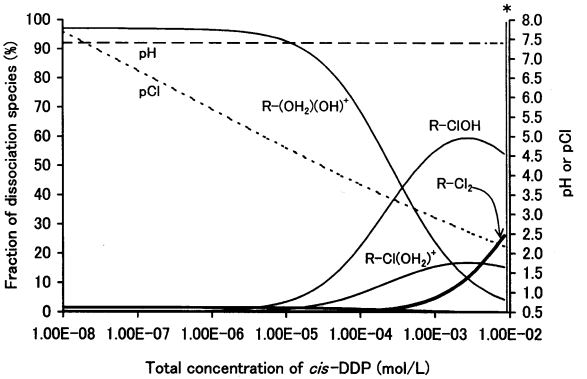

Fig. 3. Simulation of the effect of total *cis*-DDP concentration on the fraction changes of its hydrolysis and dissociation species and pCl changes in water (pH 7.4). R = $(NH_3)_2Pt$; * saturated solution at 25 °C NaCl = 0 mol/l; pH 7.4 (fixed).

and pK_2 (4.50) than pCl (7.70–6.30) in the solution. This condition is very favorable for them. As the total concentration increases more than about 3E−7 mol/l, the pH tends to decrease where the hydroxo-aqua turns to be unfavorable, shifting to the diaqua. Simultaneously, the diaqua tends to the chloro-aqua as the pCl approaches the pK_2. The maximal fraction of the diaqua was seen at about 4E−6 mol/l by the coordination of pH, pCl and pK_2. This is explained in a way that the diaqua turns less favorable as the pCl approaches pK_1—the dichloro more favorable, followed by decreasing of the chloro-aqua. Further increase of the total drug concentration decreases the pH and pCl where the chloro-aqua is most favorable at about 4E−4 mol/l mainly due to decreasing pCl produced by the drug itself.

Most in vitro experiments have been conducted in the range of total concentrations of 1E−4–1E−3 mol/l arbitrarily set up (Zheng et al., 1997; Zenker et al., 2000; Hahn et al., 2001; Heudi et al., 2001). It should be, therefore, noticed that the existing reactive species, the chloro-aqua and diaqua, drastically change in this range as a function of the total concentration. This may lead to confused interpretation of the results when removal of the reactive species and new supplies of them due to consecutive equilibria are involved.

3.2. Dissociation of cis-DDP in water at pH 7.4

Fig. 3 shows the fraction changes of respective species and pCl at a fixed pH 7.4 often set up in in vitro experiments. Although the whole picture appears to shift to the higher drug concentrations, the existing species and their fractions are different from ones in sole water. At pH 7.4 the hydroxo-aqua is only the predominant species while the other species are found almost null up to the total concentration of about 1E−5 mol/l. This is because of the contribution of pK_{a1} (5.63) and pK_{a2} (9.25) allowing the hydroxo-aqua most favorable and suppressing the formation of the dihydroxo. These processes also maintain the chloro-aqua less than 1%, resulting in null chloro-hydroxo despite pK_{a3} (6.85) in this diluted region.

As the total concentration increases more than about 1E−5 mol/l at which pCl ≈ pK_2(4.50), the hydroxo-aqua is converted to the chloro-hydroxo through the diaqua and chloro-aqua as the pCl decreases where the pK_{a1}, pK_{a3} and pK_{a2} are mainly involved.

3.3. Dissociation behavior of cis-DDP in normal saline

As shown in Fig. 4, the dichloro is stabilized in saline (0.9% w/v, pCl 0.8) where the fractions of

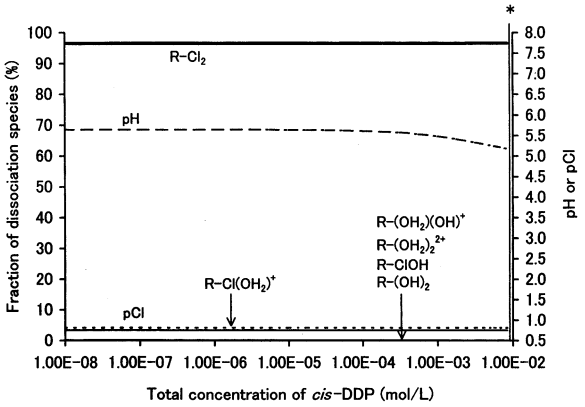

Fig. 4. Simulation of the effect of total *cis*-DDP concentration on the fraction changes of its hydrolysis and dissociation species and pH changes in normal saline. R = $(NH_3)_2Pt$; * saturated solution at 25 °C NaCl = 0.154 mol/l; initial pH of distilled water assumed: 5.64. Equilibrium constants at $\mu = 0.154$ were used.

the dichloro (97%) and the chloro-aqua (3%) remained unchanged throughout, because of largely added common ions of chloride. Although the chloro-aqua exists in only about 3% at any total concentrations, even such, low fractions may not always be negligible if the positively-charged chloro-aqua is highly reactive to some nucleophilic substances especially in the relatively higher total concentrations of *cis*-DDP. The reactive species will be supplied by new equilibria to compensate them, if it reacts with other compounds. The

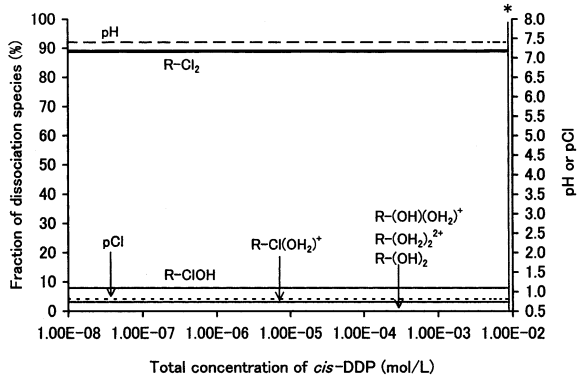

Fig. 5. Simulation of the effect of total *cis*-DDP concentration on the fraction changes of its hydrolysis and dissociation species in normal saline (pH 7.4). R = $(NH_3)_2Pt$; *, saturated solution at 25 °C NaCl = 0.154 mol/l; initial pH 7.4 (fixed). Equilibrium constants at $\mu = 0.154$ were used.

diaqua, which is considered more reactive than the chloro-aqua, is null. While the neutral dichloro was reported to directly react with L-methionine in the presence of NaCl (Goswami et al., 1989; Heudi et al., 1999), the present study may explain a variety of conflicting results by considering a chloro-aqua contribution in part to the reaction instead of the parent species. The hydroxo-aqua and dihydroxo were found null, because of higher chloride ions and lower pH maintained.

3.4. Dissociation of *cis*-DDP in normal saline at pH 7.4

Fig. 5 shows the whole picture is very similar to one in saline where the pH is not adjusted. The background high chloride concentration naturally suppresses the formation of the aquated forms. However, the pH 7.4 condition is slightly favorable for the chloro-hydroxo (8%) because of pK_{a3} (6.85) < pH 7.4, resulting in the reduction of the dichloro (89 from 97%) while the chloro-aqua (3%) remained almost unchanged. The chloro-aqua could play a role in the same manner described in the Section 3.3.

3.5. Dissociation in physiological conditions

The mechanism of action of *cis*-DDP is generally considered as follows: the drug is relatively stabilized by rich chloride ions (≈ 0.11 mol/l) in blood (Alberts et al., 1989) and is intracellularly converted to a positively-charged species, the chloro-aqua, thanks to much lower chloride ions (≈ 0.01 mol/l) after passing the cell wall. This species is believed to be responsible for binding to DNA (Sundquist and Lippard, 1990; Reedijk, 1999).

After salt-stabilized *cis*-DDP is once injected into blood, the drug will encounter very complicated environments. As far as the conditions of $[Cl^-] = 0.11$ mol/l at pH 7.4 and $\mu = 0.15$ assumed to be in blood (Alberts et al., 1989), the hydrolysis and acid dissociation behavior of the drug is not far different from one in normal saline at pH 7.4, as shown in Fig. 6. Lowered chloride concentration from 0.154 mol/l in Fig. 5 reduces the dichloro by about 4% and increases the chloro-hydroxo by

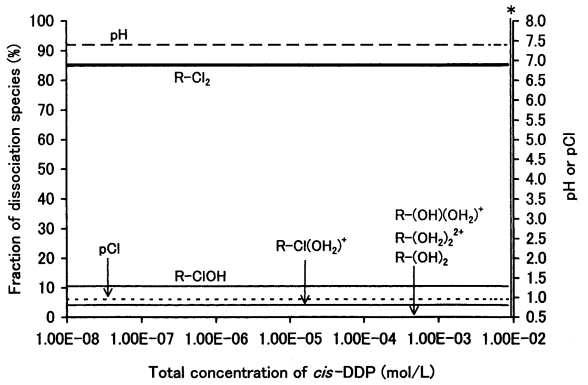

Fig. 6. Simulation of the effect of total *cis*-DDP concentration on the fraction changes of its hydrolysis and dissociation, species in assumed blood. R = $(NH_3)_2Pt$; * saturated solution at 25 °C NaCl = 0.11 mol/l; pH 7.4 (fixed). Equilibrium constants at $\mu = 0.154$ were used.

about 2%, so the integrity of the parent drug will be well maintained in the blood. It should be noted here that, in almost all therapeutical instances, the total drug concentration would be at least less than 1E−5 mol/l (Long et al., 1981) even immediately after a bolus injection.

The electrically neutral species (dichloro and chloro-hydroxo), considered available for intracellular transport, occupy about 96% of the total drug. The chloro-aqua is at about 4%, but, is possibly reactive to nucleophilic substances such as amino acids, peptides and essential enzymes in the blood and may contribute in part to adverse effects.

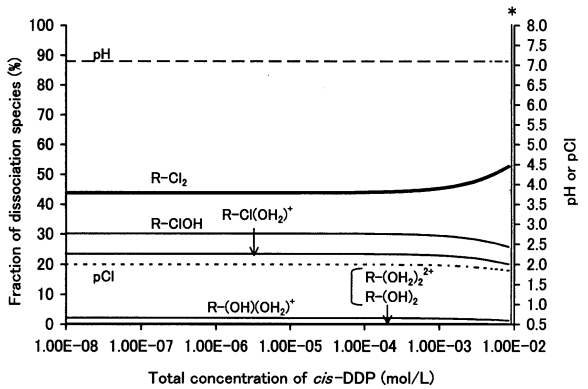

Fig. 7. Simulation of the effect of total *cis*-DDP concentration on the fraction changes of its hydrolysis and dissociation, species in assumed cytoplasm. R = $(NH_3)_2Pt$; *, saturated solution at 25 °C NaCl = 0.01 mol/l; pH 7.1 (fixed). Equilibrium constants at $\mu = 0.154$ were used.

In the intracellular low chloride condition of $[Cl^-] = 0.01$ mol/l at pH 7.1 and $\mu = 0.15$ (Alberts et al., 1989), the whole picture was naturally different from one in extracellular conditions regarding existing species, as shown in Fig. 7.

Chloride ions (pCl 2) maintain the six species constant up to the total concentration of about 1E−3 mol/l. While the intracellular drug concentration is supposed to be extremely low, the positively-charged species, considered most probably reactive to the target DNA, include the chloro-aqua (24%) hydroxo-aqua (2%), and diaqua (<1%), totaling 26%. The remnants, all neutral species, occupy at about 74%. These results were far different from a 50:50 chloro-aqua and chloro-hydroxo mixture estimated by Miller and House (1990). However, the neutral species in the blood that have crossed the cell wall may proceed towards new equilibria in the cytoplasm and such equilibria will not always be reached where the chloro-aqua reacts very rapidly with intracellular substrates.

4. Conclusion

While *cis*-DDP is dissociated in a very complicated manner in aqueous media and the simultaneous determinations of coexisting species are almost impossible on bench work, the computer simulation allowed to estimating the fraction changes of various species as a function of the total drug concentration, and the associated pH and pCl changes. Although the simulation results indicate only the fraction changes of hydrolysis and acid dissociation products at equilibria in different aqueous media, this approach will provide a rationale for re-evaluating the reactions between *cis*-DDP and various nucleophilic substances already reported elsewhere. It is worth pointing out as a prerequisite to realize what kind species prevail, especially reactive species, in terms of the total drug concentration, pH and pCl in a system concerned. As removal of reactive species takes the system to new equilibria to compensate

them, reaction kinetics should be further incorporated in the model.

Appendix A

Eqs. (13) and (14) can be expressed as a function of C_t, $[H^+]$ and $[Cl^-]$ hereafter $[H^+]=x$ and $[Cl^-]=y$.

The Newton method may be extended for numerical optimization to solve Eqs. (A.1) and (A.2).

$$f_1(x,\ y)=0 \tag{A.1}$$

$$f_2(x,\ y)=0 \tag{A.2}$$

The algorithm is as follows:

1) Let x_0, y_0 be initial approximations of the roots of Eqs. (A.3) and (A.4).
2) Calculate $f_1(x_k, y_k)$ and $f_2(x_k, y_k)$ ($k=0, 1, 2, 3, \ldots$).
3) Calculate $\partial f/\partial x$ and $\partial f/\partial y$.

$$\boldsymbol{J}(x_k,\ y_k)=\begin{bmatrix}\dfrac{\partial f_1}{\partial x} & \dfrac{\partial f_1}{\partial y}\\ \dfrac{\partial f_2}{\partial x} & \dfrac{\partial f_2}{\partial y}\end{bmatrix} \tag{A.3}$$

where $\boldsymbol{J}$ is the Jacobian matrix of derivatives.

4) Solve a set of simultaneous equations for Δx_k and Δy_k using:

$$\boldsymbol{J}(x_k,\ y_k)\begin{bmatrix}\Delta x_k\\ \Delta y_k\end{bmatrix}=-\begin{bmatrix}f_1(x_k,\ y_k)\\ f_2(x_k,\ y_k)\end{bmatrix} \tag{A.4}$$

5) Obtain the respective improved roots using:

$$\begin{bmatrix}x_{k+1}\\ y_{k+1}\end{bmatrix}=\begin{bmatrix}x_k\\ y_k\end{bmatrix}-\boldsymbol{J}(x_k,\ y_k)^{-1}\times\begin{bmatrix}f_1(x_k,\ y_k)\\ f_2(x_k,\ y_k)\end{bmatrix} \tag{A.5}$$

6) Iterate the steps 2–5 until $|(x_{k+1}-x_k)/x_k|$ and $|(y_{k+1}-y_k)/y_k|<\theta$, where θ is a designated tolerance.
7) x_{k+1} and y_{k+1} are assumed to be the roots of the equations.

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