MECHANISMS FOR REDUCTION OF MODEL AND ANTICANCER ACTIVE PLATINUM(IV) COMPOUNDS BY BIOLOGICAL MOLECULES

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To my family,

Ebstie Terefe, Samuel Kelemu, and Ellenee Kelemu

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Abstract

Electron transfer reactions of model and anticancer active platinum(IV) complexes with thiols and ascorbate ions have been investigated in a 1.0 M aqueous perchlorate medium as a function of pH and temperature using stopped-flow and conventional UV-vis spectrophotometry. Reductions of the anticancer active platinum(IV) complexes, *trans*-$[PtCl_4(NH_3)(thiazole)]$ (**1**), *trans*-$[PtCl_4(cha)(NH_3)]$ (**2**), *cis*-$[PtCl_4(cha)(NH_3)]$ (**3**), and *cis*-$[PtCl_4(NH_3)_2]$ (**4**) by glutathione and of *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**) (cha = cyclohexylamine) by a series of thiols have been studied at 25 °C in the regions $2.00 \leq pH \leq 7.00$ and $6.80 \leq pH \leq 11.22$, respectively, using an Applied Photophysics Stopped-Flow spectrophotometer. Reductions of the model platinum(IV) complexes, *cis*-$[PtCl_4(NH_3)_2]$ (**4**), *trans*-$[PtCl_4(NH_3)_2]$ (**11**), $[PtCl_6]^{2-}$ (**12**), $[PtBr_6]^{2-}$ (**13**), *trans*-$[PtCl_2(en)_2]^{2+}$ (**14**), and *trans*-$[PtBr_2(NH_3)_4]^{2+}$ (**15**) by ascorbate ions $HAsc^-$ and Asc^{2-} have been studied at 25 °C in the interval $1.75 \leq pH \leq 7.20$ using the same stopped-flow technique. Reduction of the orally active platinum(IV) dicarboxylate compounds, *cis, trans, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM216) (**7**) and *cis, trans, cis*-$[PtCl_2(OCOC_3H_7)_2(cha)(NH_3)]$ (JM221) (**8**) by ascorbate Asc^{2-} at 35 °C in the interval $7.0 \leq pH \leq 7.5$ has been investigated using a Cary 300 UV/VIS spectrophotometer. The kinetics of reduction of the geometrical isomers of compound **7**, *viz.* *trans, cis, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM394) (**9**) and *trans, trans, trans*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM576) (**10**) by Asc^{2-} and/or $HAsc^-$ at 25 °C in the interval $4.0 \leq pH \leq 7.0$ have also been studied using the stopped-flow technique in order to obtain further insight into the mechanism of reduction.

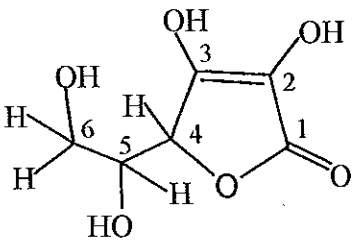
The redox reactions follow the second-order rate law: $-d[Pt(IV)]/dt = k\,[Red]_{tot}\,[Pt(IV)]$ where k denotes a pH-dependent second-order overall rate constant and $[Red]_{tot}$ the total concentration of reductant. The stoichiometeries [Pt(IV)]:[Red] for thiol (RSH) and ascorbate (Asc) reductions are 1:2 and 1:1, respectively. Reduction of the platinum(IV) complexes containing a $Cl\text{-}Pt^{IV}\text{-}Cl$ axis by the thiols and ascorbate ions is suggested to involve a reductive attack on one of such chlorides by thiolate/thiol or $Asc^{2-}/HAsc^-$ leading to the formation of a chloride-bridged transition state. Ascorbate reduction of compounds **7** and **8** which have **no** Cl-Pt-Cl axis, on the other hand, is proposed to take place *via* an outer-

sphere mechanism. The proposed mechanisms for reductions of the model and anticancer active platinum(IV) compounds by ascorbate appear to have not been reported previously.

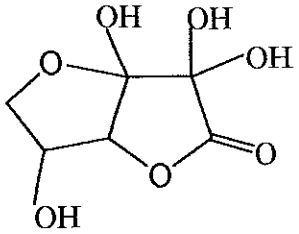
Reduction of compounds **1** – **4** by glutathione under the conditions used is very rapid while that of **5** (JM335) by the series of thiols is comparatively slow. Reduction of the oral anticancer platinum(IV) compounds **7** (JM216) and **8** (JM221) by ascorbate at the near neutral pH is more than 3 orders of magnitude slower than that of compounds **9** (JM394) and **10** (JM576). The half-life for reduction of **7** (JM216) by 5 mM total concentration of ascorbic acid (15-fold excess) at pH 7.4 and 35 °C was observed to be *ca.* 12 min. The deprotonated species RS^- (thiolate) and Asc^{2-} (ascorbate) are the predominant reductants for platinum(IV) complexes in the near neutral region.

1.0 Introduction

Platinum(IV) compounds have been known to be antitumor active since the discovery of the biological activity of cisplatin in 1969 [1]. It is almost certain that platinum(II)-based anticancer agents exhibit their activity by binding to DNA. Since Pt(IV) compounds are substitution inert, there is a general consensus that they undergo *in vivo* reduction to the more reactive Pt(II) analogs by intracellular reducing agents such as glutathione and ascorbic acid, prior to binding to DNA. This assumption is supported both by results of kinetic studies on reduction of model Pt(IV) complexes by biological molecules and by detection of Pt(II) metabolites in urine and blood samples taken from patients treated with Pt(IV)-based anticancer drugs. Knowledge of the kinetics and mechanisms for reduction of these complexes by biological reductants is thus of paramount importance in understanding the mechanism of activity and designing new complexes with suitable pharmacokinetic properties. The principal aim of this thesis work has been to investigate electron transfer reactions of model and anticancer active Pt(IV) complexes with thiols and ascorbic acid. Structures of the anticancer active compounds and the thiols used as reducing agents are shown in Charts 1 and 2, respectively. The structures of ascorbic acid and dehydroascorbic acid are shown in Fig. 1.1.

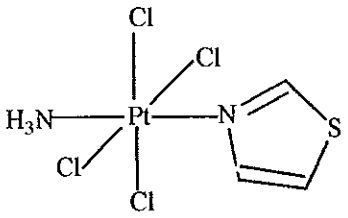


L-Ascorbic acid

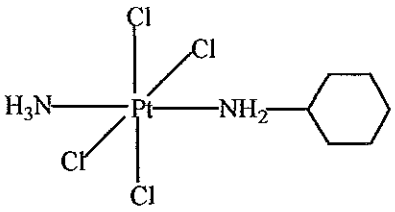


Dehydroascorbic acid
(in aqueous solution)

Figure 1.1 Structures of L-ascorbic acid and dehydroascorbic acid



1



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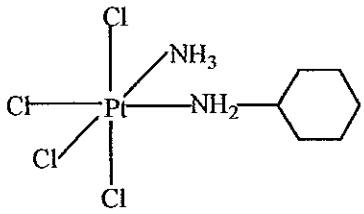

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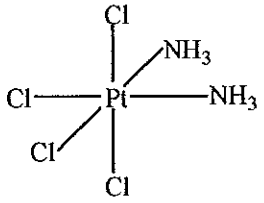

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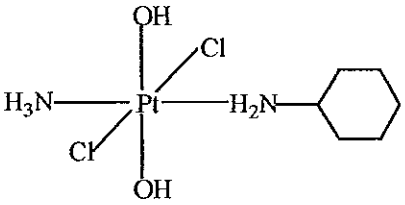
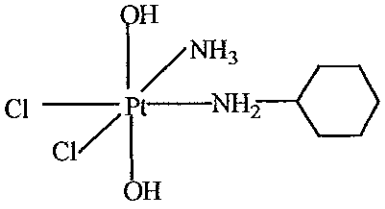
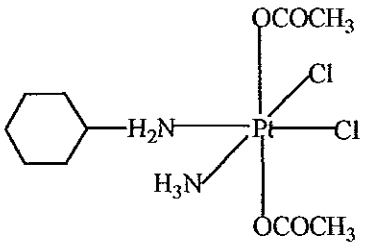


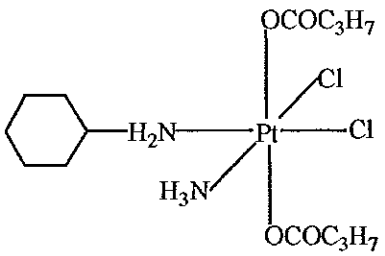
5 (JM335)



6 (JM149)



7 (JM216)

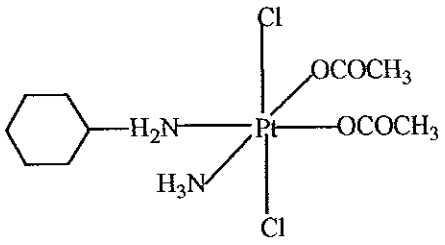


8 (JM221)

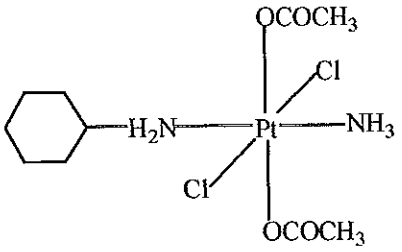
Chart 1

Chart 1 to be continued

Chart 1 continued

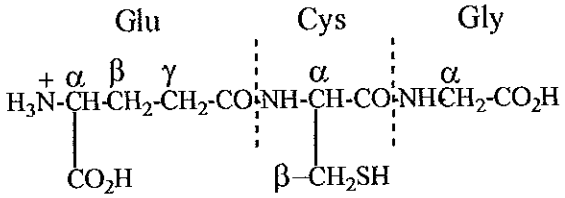


9 (JM394)

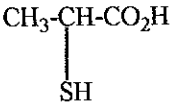


10 (JM576)

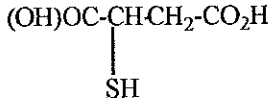
The thesis work has two major parts. The first deals with the kinetics and mechanism for electron transfer reactions of the anticancer active platinum(IV) compounds, *trans*-$[PtCl_4(NH_3)Tz]$ (**1**), *trans*-$[PtCl_4(cha)(NH_3)]$ (**2**), *cis*-$[PtCl_4(cha)(NH_3)]$ (**3**), and *cis*-$[PtCl_4(NH_3)_2]$ (**4**) with glutathione and for *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**) with thiols of disparate structural and electronic properties. Understanding the relationships between the electronic properties of the thiols and their reactivity has been an additional objective for the latter study. In the second part, reduction of model and anticancer active Pt(IV) complexes by ascorbic acid has been investigated. Temperature-dependence of the second-order overall rate constants, k, was investigated at constant pH in order to determine the activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, which are helpful in diagnosing the reaction mechanism. Ascorbic acid reduction reactions are quite complicated since it can be involved in a two-step-one-electron or a single step two-electron transfer processes. Ascorbic acid reductions of platinum(IV) chloride and bromide model complexes of different geometries and reduction potentials were investigated to delineate the reaction mechanism. A similar study has been made using the orally active platinum(IV) dicarboxylate compounds *cis, trans, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**7**) and *cis, trans, cis*-$[PtCl_2(OCOC_3H_7)_2(cha)(NH_3)]$ (**8**) and the geometrical isomers of **7**, *viz. trans, cis, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**9**) and *trans, trans, trans*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**10**).



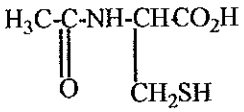
Glutathione



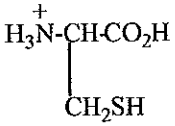
2-Mercaptopropanoic acid



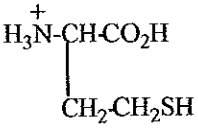
2-Mercaptosuccinic acid



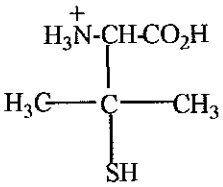
N-Acetyl-L-cysteine



L-Cysteine



DL-Homocysteine



DL-Penicillamine

Chart 2

It is hoped that the results of this study could be used both for interpreting cytotoxicity patterns of Pt(IV) anticancer compounds in relation to that of their Pt(II) counterparts as well as for developing new platinum(IV) compounds with rates of reduction suitable for uptake and distribution of the parent drug and reduction to the more reactive platinum(II) analogs, rather than passing through the body intact.

1.1 Review of the Platinum-Chemotherapy of Cancer

Cancer is a disease of uncontrolled multiplication of abnormal body cells [2]. Uncontrolled proliferation, invasiveness, and the capacity to spread are characteristics of cancer cells that are not seen in normal cells. Surgical excision, irradiation, and

chemotherapy are the three classical approaches used in the treatment of cancer. Platinum anticancer agents are special in that a heavy transition metal is involved.

1.1.1 History of the Biological Activity of *cis*-$[PtCl_2(NH_3)_2]$ (Cisplatin)

In the early 1960s, during a study of the influence of electric fields on bacterial growth, a curious phenomenon was observed [3]. When an electric field was applied across platinum electrodes immersed in an aerobic solution of *Escherichia coli* cells growing in the presence of NH_4Cl, the bacteria did not divide normally but grew into filamentous bacterial rods. Subsequent experiments by the same group soon made clear that the filamentous growth was caused by the presence of small amounts of dissolved Pt(II) and Pt(IV) compounds in the "corroding" NH_4Cl solution [4]. Detailed investigations showed that the compounds present in solution were, among others, *cis*-$[PtCl_4(NH_3)_2]$, *trans*-$[PtCl_2(NH_3)_2]$ and *cis*-$[PtCl_2(NH_3)_2]$ (Fig. 1.2). In subsequent microbiological studies the compound *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) turned out to be the most active in causing filament formation whilst its isomer *trans*-$[PtCl_2(NH_3)_2]$ (transplatin) was inactive.

1.1.2 Mechanism of Anticancer Activity

It is generally accepted that the interaction of the platinum metal center with DNA is important for the anticancer activity [5]. Evidence to confirm the role of platinum-DNA interactions came from the early observation by Rosenberg and co-workers [3] that after treatment with cisplatin cell division of *E. coli* is stopped while filamentous growth was observed. The damaged bacteria still transcribed genes and synthesized proteins and RNA suggesting that DNA synthesis was the process critically affected [4].

There is a convincing evidence that the cytotoxic properties of cisplatin are a consequence of bifunctional-DNA adduct formation [6,7]. Platinum binds to the N(7) position of purine nucleotides, resulting predominantly in 1,2-d(GPG) (65%) and 1,2-d(APG)

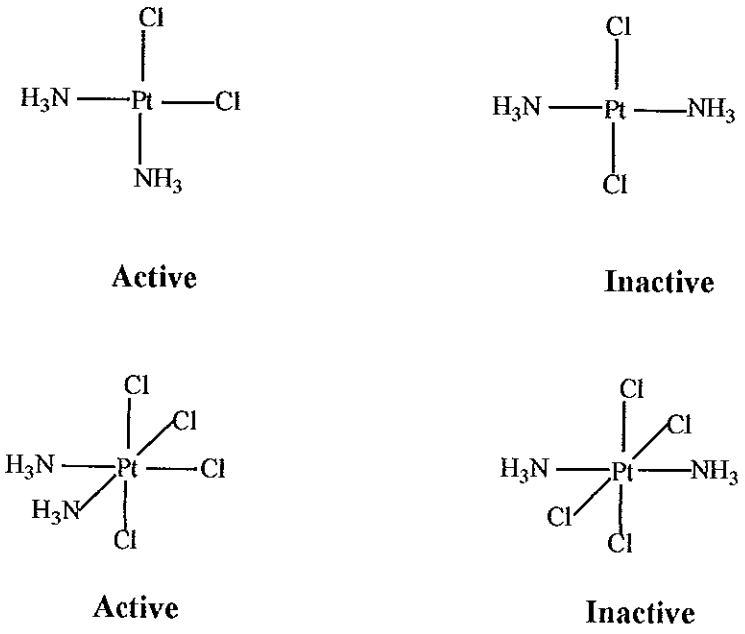


Figure 1.2 Structures of active and inactive platinum complexes

(25%) [2] intrastrand cross-links, but also in 1,3-d(GPNPG) (where N is any base) intrastrand, interstrand and protein-DNA cross-links (Fig. 1.3) [8]. Binding of cisplatin to DNA results in a local denaturation which is probably not recognized or not efficiently recognized by repair enzymes [2]. This distortion inhibits the replication process and subsequently results in the killing of the tumor cell.

Transplatin does not form 1,2-intrastrand cross-links due to its *trans* geometry [9]. It forms interstrand cross-link between the GN(7) and CN(3) of the *same* base pair, rather than the two opposite GN(7) atoms of a GC-C′G′ duplex, as for cisplatin [10]. Inactivation of the transplatin-DNA monoadduct by coupling with glutathione is several times faster than that for the cisplatin-DNA monoadduct [11,12]. In addition, transplatin itself reacts with glutathione *ca.* 300 times faster than cisplatin which might also explain the lack of activity of the former [13].

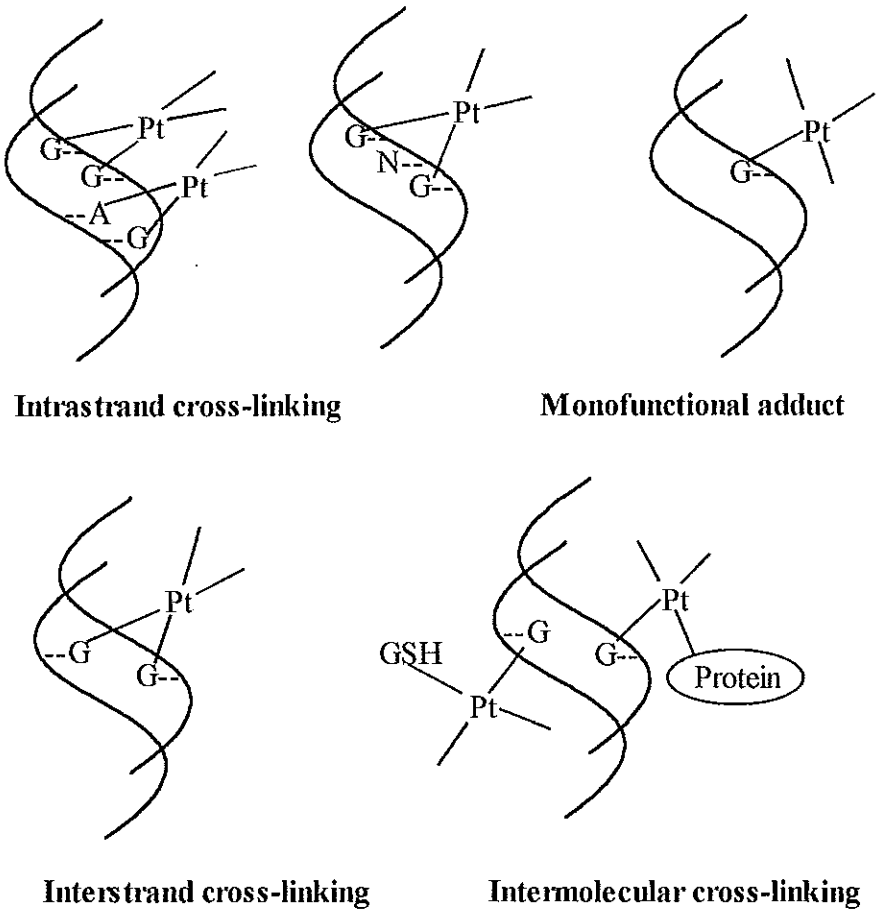


Figure 1.3 Structures of various adducts produced in DNA by cisplatin

From the structures of the purine nucleosides shown in Fig. 1.4, one can see that atoms N(3) and N(7) (and O(6) in the case of guanine) are the only sites which have free lone pairs. The lone pair electrons on N(1), N(9), and the exocyclic NH_2 groups are part of the delocalized π-electron system of the heteronuclear ring and are therefore unavailable for metal binding [14]. Because N(3) is in a sterically crowded position, with the glycosidic N(9)-C(1′) bond nearby, N(7) is the most likely binding site. Furthermore, the fact that N(7) is not involved in *Watson-Crick* base pairing, and is exposed in the major groove of the DNA duplex, makes it an even more attractive target for binding of platinum [14].

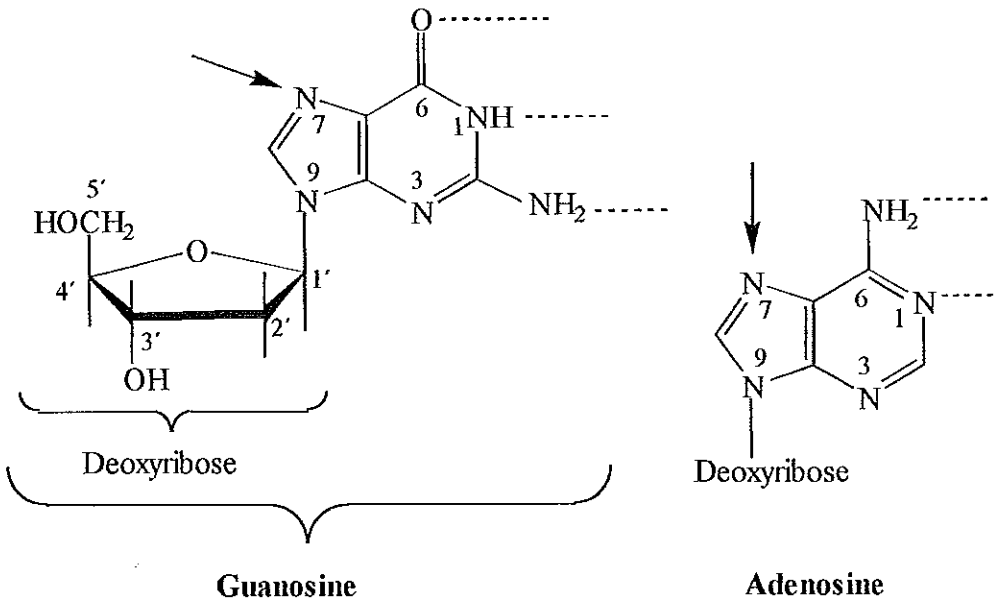


Figure 1.4 Purine nucleosides

1.1.3 Hydrolytic Activation of Cisplatin

In the earliest biological experiments, an initial insensitivity to cisplatin, lasting for about 2 h, was noted [15]. It was thus suggested that cisplatin may not itself be the active species, but rather is converted over a period of time to the actual drug. Many have reported that the first step of cisplatin binding to DNA is controlled by the rate of hydrolysis of the drug [16-20]. The rate constant for the hydrolytic replacement of the first chloro ligand at 37 °C is 7 x 10^{-3} min^{-1}, corresponding to a half-life of 2 h [21]. Hydrolysis of the second chloride ligand is about two times slower.

$$cis\text{-}[PtCl_2(NH_3)_2] = cis\text{-}[PtCl(OH_2)(NH_3)_2]^+ + Cl^- \quad , t_{1/2} = 2\ h \qquad (1.1)$$

$$cis\text{-}[PtCl(OH_2)(NH_3)_2]^+ = cis\text{-}[Pt(NH_3)_2(OH_2)_2]^{2+} + Cl^- \quad , t_{1/2} = 4\ h \qquad (1.2)$$

Hence, *cis*-$[PtCl(OH_2)(NH_3)_2]^+$ is the biologically important species; the firmly bound ammine ligands are responsible for the activity as well as for the selection of target molecule [22]. Bancroft *et al.* have reported that the rate of hydrolysis of the first chloride ion is indeed the rate-determining step for initial binding of cisplatin to DNA [11].

In blood plasma, where the chloride ion concentration is about 100 mM [23], the predominant species is the neutral dichloro complex, which can passively diffuse across cell membranes. Inside the cell, the chloride ion concentration drops to about 4 mM, allowing various hydrolysis products to be formed and interact with intracellular biomolecules such as RNA and glutathione besides binding to DNA [2].

1.1.4 Classical Structure-Activity Relationships (SARs)

The first relationship between structure and activity emerged from the initial studies by Rosenberg and co-workers who found that the *cis* isomers of both $[PtCl_2(NH_3)_2]$ and $[PtCl_4(NH_3)_2]$ interfered with the cell division in *E. coli* while the *trans* isomers were inactive [24]. Soon after, Cleare and Hoeschele confirmed that complexes with the *trans* geometry were inactive and added other criteria [25]. Most of the platinum compounds with antitumor properties fulfill the following structure requirements.

(i) A *cis* geometry with the general formula *cis*-$[PtX_2(amine)_2]$ for Pt(II) compounds and *cis*-$[PtY_2X_2(amine)_2]$ for Pt(IV) compounds.

(ii) The X ligand should be an anion with intermediate binding strength such as chloride or oxalate. Y in case of Pt(IV) can be chloride, hydroxide, or carboxylate ligands oriented *trans* to each other.

(iii) The amine ligands should possess at least one NH moiety, necessary for hydrogen bonding interactions [26]. Hydrogen bonding interactions are important in stabilizing the 1,2-d(GPG) intrastrand adduct [27].

1.1.5 Cisplatin Derivatives

Cisplatin is widely used for the treatment of bladder, ovarian, and testicular tumors [26,28]. However, the clinical effectiveness of this drug is greatly affected by its undesirable side effects (including renal damage, loss of hearing of high frequency, bone marrow suppression, neurotoxicity, and severe nausea and vomiting [29].

It is hypothesized that part of the nephrotoxicity, and possible bone marrow suppression induced by platinum-based antitumor agents may involve ligand exchange reactions of platinum with sulfhydryl groups and subsequent inactivation of essential enzymes and other proteins [16,30,31]. Platinum complexes have been shown to react with plasma protein sulfhydryl groups [32] and with methionine. For instance, a *bis* adduct of the latter has been isolated from urine samples of patients receiving cisplatin [33].

Drug resistance is another major obstacle for the successful treatment of cancer with cisplatin. The different mechanisms that account for this phenomenon include, a) decreased platinum accumulation, b) elevated levels of glutathione which sequester cisplatin before it reaches its pharmacological target, and c) enhanced repair capacity of cells to remove Pt-DNA lesions [34].

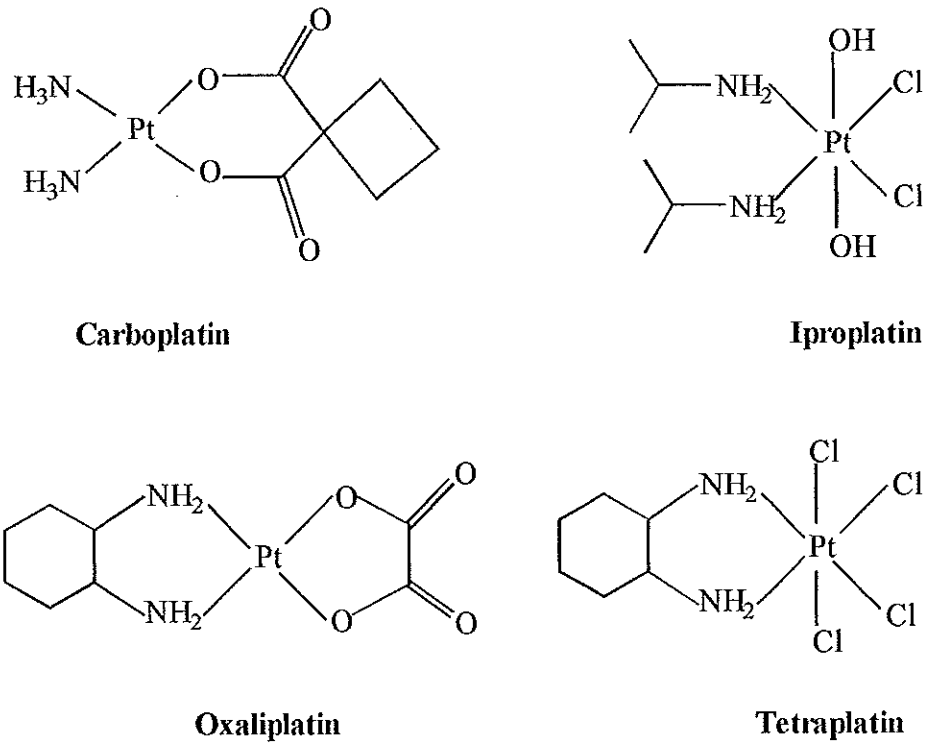


Figure 1.5 Structures of some second-generation platinum anticancer active compounds

As a consequence of the undesirable side effects, the search for the so-called "second-generation" complexes has concentrated on designing more effective and less toxic cisplatin analogues [35]. It was hypothesized that modification of cisplatin to contain less labile leaving groups could alter toxicity. The compound *cis*-1,1-dicarboxylatocyclobutane-diammineplatinum(II) (carboplatin) (Fig. 1.5) was developed as a second-generation drug which is now in worldwide use as an anticancer active agent [36].

Carboplatin exhibits markedly reduced side effects while maintaining a spectrum of clinical activity similar to that of cisplatin. Bone marrow suppression, which is not usually severe with cisplatin, is the dose-limiting toxicity of this drug [37]. The reduced side effects of carboplatin are attributed to its lower reactivity rendered by the chelating 1,1-dicarboxylatocyclobutane (CBDCA) ligand. Carboplatin shows a high degree of cross-resistance with cisplatin implying that the two drugs have similar DNA adduct profiles [38]. The fact that the drug reacts faster with 5′-GMP (5′-guanosine monophosphate) than with nitrate, phosphate and chloride ions suggests that direct attack of carboplatin on DNA may take place [39].

Another most successful cisplatin analogue which was developed in France [40-42] is [Pt(oxalate)(dach)] (oxaliplatin) (Fig. 1.5). Oxaliplatin has less nephrotoxicity than cisplatin, presumably related to its decreased reactivity. The dose-limiting toxicity of the drug is sensory neuropathy, which is a characteristic feature of all dach-containing platinum derivatives [42,43]. Its DNA adducts are similar to those of cisplatin and carboplatin [44]. The idea of circumventing drug resistance by modifying the carrier ligands is demonstrated by the *in vivo* activity of oxaliplatin in cisplatin-resistant tumor cells [41]. The major difference between the diammine-based drugs, cisplatin and carboplatin, and dach-containing derivatives is likely to be in the manner in which cellular repair enzymes recognize and process Pt-DNA adducts [41].

Since platinum(IV) complexes have octahedral geometry it was hoped that these complexes, due to their structural difference from square planar platinum(II) complexes,

could be used to circumvent drug resistance. Pt(IV) compounds are also advantageous because they have a better water solubility than their Pt(II) analogues. The platinum(IV) compounds *cis, trans, cis*-[$PtCl_2(OH)_2$(*i*-prNH_2)$_2$] (iproplatin) and *cis*-[$PtCl_4$(dach)] (tetraplatin) have been developed as second-generation cisplatin Pt(IV) analogues (Fig. 1.5). The development of dach-containing platinum complexes including tetraplatin originated largely through the demonstration of their activity against cisplatin-resistant murine *L*1210 and *P*388 leukemia tumour cells [45,46] and lack of nephrotoxicity [47,48]. Although both iproplatin and tetraplatin have reached the stage of clinical trials, the former failed to display activity in its Phase-II trials [48] and the latter was withdrawn from a Phase-I trial because it caused severe neurotoxicity [49].

1.1.6 Development of Orally Active Platinum(IV) Complexes

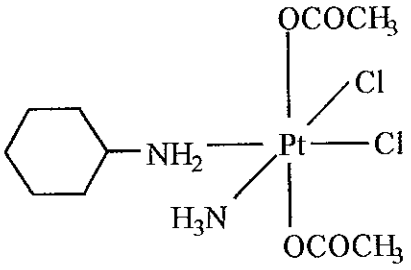
Both cisplatin and carboplatin are given by intravenous injection and have been found to be poorly absorbed by the gastrointestinal tract when given orally [49]. Besides, a brief clinical study revealed that carboplatin when given orally results in severe gastrointestinal side effects [50]. The search for orally active platinum drugs has thus become an area of advancing cancer chemotherapy distinct from other second- and third-generation drug development programmes.

A novel class of platinum antitumor compounds, so-called Pt(IV) mixed-amine dicarboxylates, has been developed by Johnson Matthey, Institute of Cancer Research and Bristol Myers Squibb specifically to circumvent the poor gastrointestinal absorption of cisplatin and carboplatin. Mixed-amine complexes offer the prospect of improved solubility due to reduced lattice energies in the solid state when compared with the *bis*-ammine series [51]. These compounds can be given orally and display excellent antitumor activity *in vivo* and in animal tumor models [52,53]. Mixed-amine Pt(IV) dicarboxylate compounds are suitable for oral administration because they are neutral, kinetically inert, acid-stable and

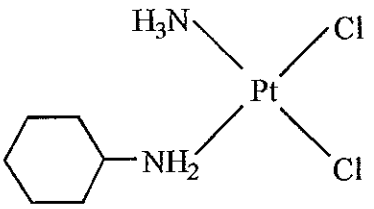
lipophilic. Lipophilicity is, in fact, considered to be important for optimizing uptake through the intestine.

Since the amine ligands are essential for activity, modification of these groups to promote oral absorption is likely to affect the activity of the complexes. Platinum(IV) complexes were chosen because they offered additional sites for altering lipophilicity of the carboxylate ligands. The synthesis of these complexes is a challenge, however, since platinum(IV) complexes are substitution inert. This problem has been overcome by exploiting the inertness of the metal-ligand bonds. The oxygen atom of the hydroxide ligand retains strong nucleophilic character after bonding to Pt(IV), allowing it to participate in reactions with suitable electrophiles while the inertness of the bond to the metal ensures that the complex remains intact [54]. A reaction with much greater applicability is that of the dihydroxo Pt(IV) complex with an acid anhydride rather than with the corresponding organic acid. This reaction allowed the preparation of the mixed-amine platinum(IV) complexes containing carboxylate ligands with a wide range of lipophilicity.

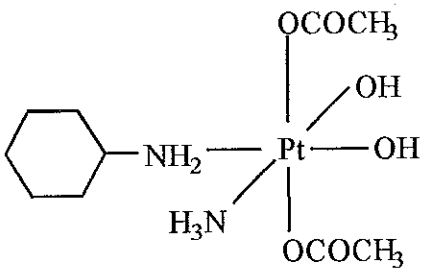
The compound selected for clinical evaluation from the mixed-amine platinum(IV) complexes was *cis, trans, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM216) (Fig. 1.6). Its selection was on the basis that it possesses both good oral antitumor activity against a variety of tumor models, displays a low emesis score in the ferret model and favorable physicochemical properties [55]. JM216 and the platinum(IV) complex containing a longer-chain dicarboxylate, *viz. cis, trans, cis*-$[PtCl_2(OCOC_3H_7)_2(cha)(NH_3)]$ (JM221) circumvent drug resistance related to reduced platinum accumulation but showed only partial circumvention against tumor where resistance is due to enhanced Pt-DNA repair/tolerance to Pt-DNA adducts [52,56]. JM216 is transported across plasma membranes through passive diffusion, predominantly as a result of its enhanced lipophilicity compared to cisplatin [57]. In common with cisplatin, JM216 is capable of forming both interstrand and intrastrand cross-links [58], presumably after reduction to *cis*-$[PtCl_2(cha)(NH_3)]$ (JM118).



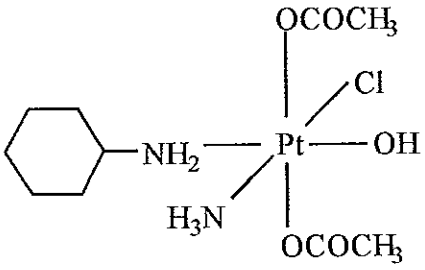
JM216



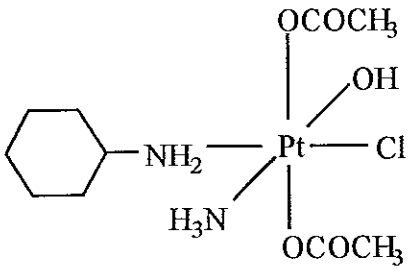
JM118



JM383



JM518



JM559

Figure 1.6 Structure of JM216 and its biotransformation products

1.1.7 Non-Classical Platinum Anticancer Active Complexes

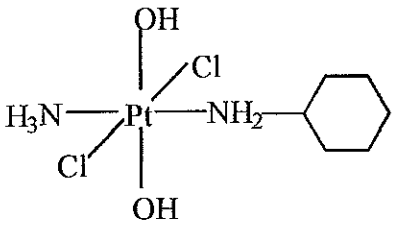
As an alternative approach to circumvent the problem of drug resistance, there has been a renewed interest in attempts to activate *trans* platinum complexes [59-63]. The rationale behind the design of these platinum complexes is that their structural features would

result in binding to DNA in a manner distinct from that of cisplatin [64]. Activation of the *trans* geometry breaks the paradigm for structure-activity relationships. These complexes fall into three categories: 1) *trans*-[$PtCl_2(L)(L')$] (L and/or L'= pyridine-like ligands), 2) *trans*-[$PtCl_2(OH)_2(L)(L')$] (L, L'= ammine, amine), and 3) *trans*-[$PtCl_2L_2$] (L = iminoether).

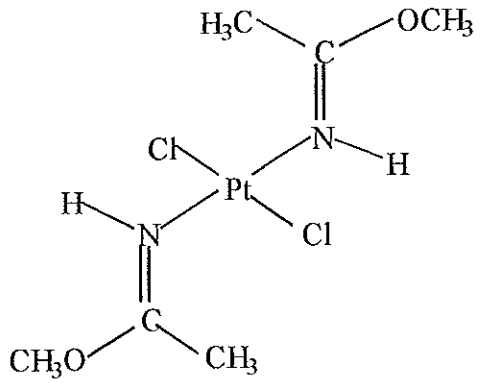
A fundamental difference between the chemistry of cisplatin and transplatin is that the latter is kinetically more reactive than the former, which may explain its lack of activity. Farrell reasoned that the substitution reactivity of platinum complexes with the *trans* geometry could be retarded by replacing NH_3 by sterically demanding ligands such as pyridine [60]. A further decrease in kinetic lability and increase in solubility is expected from Pt(IV) analogues.

Replacement of a single NH_3 of transplatin by quinoline to give *trans*-[$PtCl_2(NH_3)$-(quin)] leads to a dramatic increase in activity and indeed a Pt(IV) variant of this complex, *viz. trans, trans, trans*-[$PtCl_2(OH)_2(NH_3)$(quin)], has *in vivo* activity approaching that of cisplatin [65]. These complexes have increased cell-membrane permeability and enhanced interstrand cross-linking ability. They display cytotoxicity in both murine and human tumor cell models equivalent to cisplatin and retain activity in cells resistant to cisplatin [59,60,66]. *In vivo*, the complexes *trans*- [$PtCl_2(NH_3)$(quin)] and *trans*-[$PtCl_2(NH_3)$Tz] are marginally and moderately active, respectively, while *trans*-[$PtCl_2(py)_2$] is inactive [59,60]. It was considered likely that the lack of *in vivo* activity of *trans*-[$PtCl_2(py)_2$] is due to a decreased bioavailability of the complex, which is very sparingly soluble in water. Factors such as aqueous solubility, tissue distribution, and/or metabolic deactivation influence *in vivo* experiments.

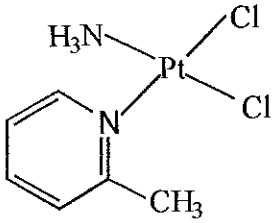
The platinum(IV) oxidation state is proved to be very useful for conferring antitumor activity on platinum complexes in which the leaving ligands have *trans* geometry [63]. Many of the *trans* platinum(IV) complexes studied both *in vitro* and *in vivo* using murine and human tumor models exhibited comparable potency to cisplatin and also overcome acquired resistance, which was mainly due to either reduced drug uptake or to enhanced removal of the



JM335



Trans-EE



AMD473

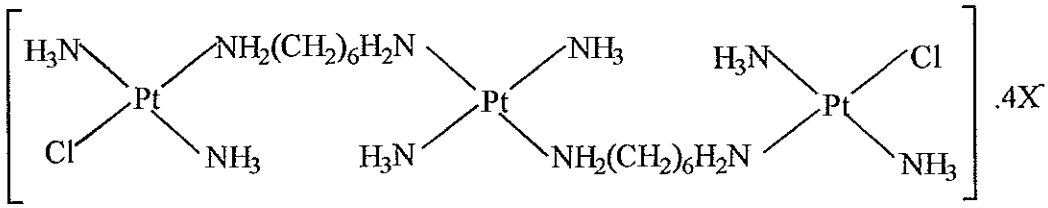


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Figure 1.7 Platinum compounds with promising preclinical and clinical activity

Noteworthy, sterically demanding carrier ligands have also been used to potentiate the antitumor activity of *cis*-oriented Pt(II) species. The compound *cis*-[$PtCl_2(NH_3)$(2-methylpyridine)] (AMD473) (Fig. 1.7) has been selected for clinical trials since it has reduced reactivity with sulfur ligands, unique DNA binding properties, and the ability to circumvent several of the major resistance mechanisms manifested by cisplatin resistant cells [74,75].

metabolite, *viz. cis*-[$PtCl_2$(cha)(NH_3)] (JM118), 6 h after administration, is considered to be an indicator of a good therapeutic efficacy [87]. It is important for a Pt(IV) drug to have a reduction rate which is an effective compromise between the Pt(IV) state for uptake and distribution of the parent drug and reduction to Pt(II) sufficiently rapidly to achieve reaction with DNA rather than being excreted intact.

The cytotoxicity of JM216 has been observed to be influenced by the levels of glutathione [89]. Increased glutathione levels are a common feature in cellular resistance against platinum drugs [90] and levels of glutathione have been shown to correlate with sensitivity to platinum agents in human and murine tumor cells [91,92]. It has been reported that when glutathione levels are low, more biotransformation products are formed inducing higher toxicity [89]. Trapping by glutathione reduces both the number and the relative amount of parent, and cytotoxic metabolites, thereby decreasing cytotoxicity.

To date, there has been no report on the nature of the biotransformation products of JM335 except that *trans*-[$PtCl_2$(NH_3)(cha)] (JM334) is presumed to be a likely metabolite [93]. What is intriguing in this regard is the lack of *in vivo* activity of JM334 while its Pt(IV) counterpart, *i.e.*, JM335 is reported to be active [67]. This observation tend to invalidate the theory that platinum(IV) complexes are activated *in vivo* by reduction to the Pt(II) oxidation state. It might be possible, however, that platinum(IV) complexes, by way of different cell transport properties, display better anticancer activity than their platinum(II) analogues [94]. Of particular interest is the fact that JM335 is less effective than its *cis* congener JM149 in overcoming resistance in cells possessing significantly elevated levels of glutathione [63]. The observed difference might be attributable to difference in reactivity of these compounds towards reduction by glutathione. Further investigations are needed to find out whether an alternative metabolite, possibly *trans*-[$Pt(OH)_2$(cha)(NH_3)], or the properties of the parent drug are important for cytotoxicity toward cisplatin-resistant cells.

1.2 Mechanisms of Electron Transfer Reactions of Transition Metal Complexes

1.2.1 General

In the broader sense, inorganic reactions are classified as either substitution or electron transfer. The latter reactions are accompanied by a net change of the oxidation state of the reaction partners. If the change in oxidation state of the reducing agent is the same as that of the oxidizing agent, the reaction is termed as complementary. If the change in oxidation state of the reducing agent is different from that of the oxidizing agent, the redox reaction is termed as noncomplementary.

Although this classification has no direct mechanistic implications, complementary reactions, at least qualitatively, are faster than non-complementary reactions [95]. Two mechanistic possibilities for electron transfer reactions of transition metal complexes are distinguished: outer-sphere and inner-sphere mechanism.

1.2.2 Outer-Sphere Electron Transfer

During outer-sphere electron transfer, the coordination sphere of the oxidant and reductant remain intact. The reactants are considered as hard spheres and an electrostatic approach can be used to anticipate the rates of electron transfer. Rate constants for outer-sphere electron transfer reactions are principally correlated with the driving force ΔE of the reactions. A mechanistic decision between outer- and inner-sphere electron transfer is often based on whether or not the reaction rate corresponds reasonably to the predictions of the outer-sphere theory [96]. This theory predicts the existence of correlation of rate constants with those for the reduction of the same substrate by known outer-sphere reductants. Lack of correlation is usually taken as an indication for the operation of inner-sphere mechanism.

The *Frank-Condon principle* is fundamental to the outer-sphere theory. The principle states that electron movement is much faster than nuclear motion; thus internuclear distances do not change during the instant of electron transfer. In order to satisfy this principle, it is assumed that on approaching the transition state, the bond lengths of the reactants will adjust

to approach those of the products. What is often called the simplified Marcus cross relationship is given by Eqn. (1.3) where f_{12} is defined by Eqn. (1.4). The parameters

$$k_{12} = \sqrt{k_{11}k_{22}K_{12}f_{12}} \tag{1.3}$$

$$\log f_{12} = \frac{(\log K_{12})^2}{4\log\left(\frac{k_{11}\,k_{22}}{Z^2}\right)} \tag{1.4}$$

k_{12} and K_{12} are the rate and equilibrium constants, respectively, for the cross reaction between the oxidant and reductant (the redox partners are arbitrarily designated by 1 (oxidant) and 2 (reductant)) . The parameters k_{11} and k_{22} are the self-exchange rate constants for oxidant and for reductant, respectively, and Z is the collision number for the reactants in solution ($\approx 10^{11}$ M^{-1} s^{-1}). $f_{12} \approx 1$ for most practical purposes, unless K_{12}, which is defined by Eqn. (1.6), is large. Eqn. (1.3) is therefore further simplified to yield Eqn. (1.5).

$$k_{12} = \sqrt{k_{11}k_{22}K_{12}} \tag{1.5}$$

$$\ln K_{12} = nF(E^{\circ}_{11} - E^{\circ}_{22})/RT \tag{1.6}$$

The parameters E°_{11} and E°_{22} in Eqn. (1.6) represent the reduction potentials for the self-exchange reactions of the reactants 1 and 2, respectively. The quantities F, R and T are the Faraday's constant, universal gas constant and the absolute temperature, respectively. For a one-electron transfer (n = 1) at 25 °C, the logarithmic form of Eqn. (1.5) is given by

$$\log k_{12} = 0.5[\log k_{11} + \log k_{22}] + 0.5\,(F/RT)\Delta E^{\circ} \tag{1.7}$$

Where ΔE° is the reduction potential in volts. Eqn. (1.7) is applied to calculate one of the self-exchange rate constants or the cross-reaction rate constant k_{12} in order to compare it to an experimental value. A good correlation between experimental and calculated rate constants indicates the operation of an outer-sphere electron transfer mechanism.

From a practical point of view, outer-sphere mechanism is proposed if the rate constant for electron transfer is larger than the rate of ligand substitution on either metal. It is

almost certain that this mechanism is operative if the partners of a redox reaction are quite inert to substitution. Also, outer-sphere electron transfer is generally thought to be the mechanism when all the ligands coordinated to both metal centers have no unshared electron pairs.

Electron transfer by outer-sphere mechanism consists of three steps: (i) formation of a precursor ion-pair complex, (ii) irreversible electron transfer, and (iii) dissociation of the successor complex to yield the products. The actual electron transfer process is rate controlling since the precursor formation and successor dissociation are both diffusion controlled. This is so since there are no bonding interactions between partners in precursor and successor complexes. Saturation of the observed rate constant is one feature characteristic of outer-sphere electron transfer. For instance, the pseudo-first-order rate constant, k_{obsd}, for reduction of $[Co(NH_3)_5py]^{3+}$ by large excess of $[Fe(CN)_6]^{4-}$ is defined by Eqn. (1.8) where K_{os} is the outer-sphere complex formation constant and k_{et} the intramolecular electron transfer rate constant [97]. As can be seen from Eqn. (1.8), the value of k_{obsd} does not increase beyond a limiting value of k_{et} by increasing $[Fe(CN)_6^{4-}]$ when the condition

$$k_{obsd} = \frac{k_{et} K_{os} \left[Fe(CN)_6^{4-}\right]}{1 + K_{os} \left[Fe(CN)_6^{4-}\right]} \qquad (1.8)$$

$K_{os}\,[Fe(CN)_6^{4-}] >> 1$ is satisfied. The usual first order dependence of k_{obsd} on $[Fe(CN)_6^{4-}]$ is observed for the condition $K_{os}\,[Fe(CN)_6^{4-}] << 1$.

In qualitative terms, the rate of an outer-sphere redox reaction is determined by the electron donating strength of the reducing agent and the electron accepting strength of the oxidizing agent [98]. Coordination of ligands with strong electron pair donor properties leads to a decrease in oxidizing and to an increase in reducing properties. The donicity of ammonia, for instance, is higher than that of pyridine and hence ammonia complexes are reduced at lower rates than the pyridine analogues (Table 1.1) [99-101]. In a similar way

$[Co(NH_3)_6]^{3+}$ is not reduced as rapidly as $[Co(NH_3)_5(OH_2)]^{3+}$ since the donicity of ammonia is greater than that of water [100,102].

Table 1.1 Rate constants for outer-sphere oxidation of $[Cr(OH_2)_6]^{2+}$ by Co(III) and Ru(III) am(m)ine complexes[¶]

Oxidant	$k/M^{-1}\ s^{-1}$
$[Co(NH_3)_6]^{3+}$	8.9×10^{-5}
$[Co(NH_3)_5py]^{3+}$	4.3×10^{-3}
$[Ru(NH_3)_6]^{3+}$	2.0×10^{2}
$[Ru(NH_3)_5py]^{3+}$	3.4×10^{3}

[¶] refs 99-101

What is more interesting about the oxidation of $[Cr(OH_2)_6]^{2+}$ by the complexes in Table 1.1 is that the Ru(III) complexes are about six orders of magnitude more reactive than the Co(III) analoges. This striking difference in rate is attributed to the fact that the Ru(III) complexes have much higher self-exchange rate constants than the corresponding Co(III) complexes. The rate constants for the self-exchange reactions of the hexaammine complexes, for instance, are 10^{-7} [103] and $2.2 \times 10^{4}\ M^{-1}\ s^{-1}$ [104] for $[Co(NH_3)_6]^{3+/2+}$ and $[Ru(NH_3)_6]^{3+/2+}$, respectively.

Pentaamminecobalt(III) complexes are suitable for studying electron transfer reactions since they are inert to substitution reactions. The inertness of these complexes allows one to vary the six ligand and investigate features of the reactions that depend on the nature of the substituent ligand. For instance, the rate of outer-sphere reduction of $[Co(X)(NH_3)_5]^{2+}$ (X = F, Cl, Br, I) by $[Cr((bipy)_3]^{2+}$ increases in the order F< Cl < Br < I [100,102]. This trend of reactivity is explained in terms of the relative "permeability" of the halide ions to electron flow from Cr(II) to Co(III) [100,105].

1.2.3 Inner-Sphere Electron Transfer

In this sort of mechanism, electron transfer is preceded by substitution of a ligand from one reactant into the coordination sphere of the other to form a bridged complex. The net electron transfer process can be considered as occurring in four steps: (1) loss of one or more ligands from the *labile* partner, (2) formation of a bridged precursor complex, (3) electron transfer, and (4) subsequent dissociation of successor complex into the products. The successor complex dissociates according to the relative stability of the possible products. The atoms or groups forming the bridge generally prefer the metal center of higher oxidizing properties and hence a ligand transfer from the reduced to the oxidized partner is frequently observed [105]. A classical example for inner-sphere mechanism involving atom transfer is one given in Eqn. (1.9).

$$[CoCl(NH_3)_5]^{2+} + Cr^{2+}(aq) + 5\,H^+ \rightarrow Co^{2+}(aq) + CrCl^{2+} + 5NH_4^+ \qquad (1.9)$$

Identification of a product to which the bridging group has been transferred is a compelling evidence for inner-sphere mechanism. The oxidation product has to be reasonably stable, however, for this method of making mechanistic decision to be useful. By exploiting the substitution-inert property of the oxidized Cr(III) product, Taube and Myers [105] were able to demonstrate that reduction of $[CoCl(NH_3)_5]^{2+}$ proceeds by an inner-sphere activated complex accompanied by ligand transfer of the chlorine from cobalt to chromium, producing $[CrCl(OH_2)_5]^{2+}$.

Another characteristic feature of inner-sphere reactions is the sensitivity of the rates to the nature of the bridging ligands. More commonly, this mechanism is inferred if the electron transfer is unusually fast. Reduction of $[Co(NH_3)_6]^{3+}$ by $[Cr(OH_2)_6]^{2+}$ follows an outer-sphere mechanism with a rate constant k of 10^{-4} M^{-1} s^{-1} [106]. Replacement of only one of the six ammonia ligands in $[Co(NH_3)_6]^{3+}$ with chloride accelerates the rate of reduction by a factor of over nine orders of magnitude, suggesting a changeover of mechanism from outer- to inner-sphere electron transfer. A wide range of bridging ligands is known, such as the halide ions, hydroxide ion, and carboxylate ions. Ligands such as ammonia, bipyridyl, and *o*-

phenanthroline cannot act as bridging groups because of lack of unshared electron pairs, which are necessary for simultaneous bonding to two metal centers.

For inner-sphere electron transfer reactions of the type shown in Eqn. (1.10), the

$$[ML_6]^{m+} + [M'Y_6]^{n+} \underset{}{\overset{K}{\rightleftharpoons}} [L_5M\text{-}L\text{-}M'Y_5]^{(m+n+1)+} \xrightarrow{k_{et}} \text{Products} \qquad (1.10)$$
$$Y^-$$

experimental rate constant k_{exp} is a product of the equilibrium constant K for formation of the precursor complex and the electron transfer rate constant k_{et}. Several kinetic trends can be interpreted as largely reflecting changes in K. Reduction of $[Co(X)(NH_3)_5]^{2+}$

$$k_{exp} = K\, k_{et} \qquad (1.11)$$

(X = F, Cl, Br) by $[Cr(OH_2)_6]^{2+}$, for instance, decreases in the order $Br^- > Cl^- > F^-$ [107], but the opposite trend of reactivity is observed when Eu^{2+} is the reducing agent [102]. This is rationalized from the knowledge that Eu^{2+} is a harder acid and forms stronger complexes with F^- whereas Cr^{2+} forms stronger complexes with Br^-. If X is a carboxylate anion, then the order of reactivity of $[Co(X)(NH_3)_5]^{2+}$ with $[Cr(OH_2)_6]^{2+}$ is $HCO_2^- > CH_3CO_2^- > CHCl_2CO_2^- > CF_3CO_2^- > (CH_3)_2CCO_2^-$ [108]. A combination of steric and electron withdrawing effects causing K to decrease for the bridged intermediate explain the observed difference in reactivity.

1.2.4 Reduction of Transition Metal Complexes by L-Ascorbic Acid

L-Ascorbic acid (Fig. 1.1) is a diprotic organic molecule found naturally in a wide variety of plants and animals [109]. It is not produced by the human body and the only source is from diet. The role of ascorbic acid (Vitamin C) in the cure and prevention of scurvy is well studied and documented [110]. A fundamental feature of the chemistry of L-ascorbic acid is its redox behaviour. It is, in fact, reported to be an outstanding antioxidant in human blood plasma [111]. Much of the loss of Vitamin C from food and drink is due to its

oxidation by dissolved oxygen. This, so-called "auto-oxidation" of ascorbic acid, is a complex process involving catalysis by transition metal ions such as Fe(III) and Cu(II) [112-115].

The first and second acid dissociation constants, ($H_2Asc = HAsc^- + H^+$, K_1) and ($HAsc^- = Asc^{2-} + H^+$, K_2), for ascorbic acid measured under various conditions of ionic strength and temperature have been reported (Table 1.2) [116-118]. Depending on the nature of the metal complex and pH of the reaction medium, either the undissociated molecule (H_2Asc), hydrogen ascorbate ($HAsc^-$) or ascorbate (Asc^{2-}) could be the dominant reducing species. Ascorbic acid reduction reactions could be classified into three categories [119]: (i) outer-sphere, (ii) inner-sphere, and (iii) inner-sphere electron transfer in which ascorbate is bound at a ligand of the complex prior to electron transfer.

Ascorbic acid is a well-characterized two-electron reductant which in principle has the capacity to enter into both one- and two-electron transfer reactions [120-122]. Kinetic studies on oxidation of ascorbic acid by and large involved strong outer-sphere one-electron acceptor complexes in aqueous acidic medium. Particular interest has been devoted to metal ion-catalyzed oxidation of ascorbic acid by dioxygen and, among others, Fe(III) complexes have received large attention [122-125]. For instance, oxidation of ascorbic acid in acidic perchlorate medium, $[H^+]$ = 0.3 - 1.0 M, by various substituted Fe(III)-1,10-phenanthroline complexes, $[FeL_3]^{3+}$ (L = phen, nphen, cphen, mphen, mphen, see List of Abbreviations) has been reported to proceed by an outer-sphere mechanism [122]. A mechanistic decision on such a redox system is not difficult since the oxidants are substitution inert and do not possess vacant co-ordination sites. Linear correlation of the rate constants with the formal reduction potentials of the $[FeL_3]^{3+}/[FeL_3]^{2+}$ couples is taken to be a strong support for the proposed mechanism (Fig. 1.8).

Table 1.2 Acid dissociation constants of ascorbic acid in 1.0 M aqueous $NaClO_4$ and at various temperatures[¶]

T/°C	$10^5 K_1$/M	$10^{12} K_2$/M
10.4	8.81	3.63
18.5	9.91	4.47
25.0	10.90	5.75
32.5	12.20	7.94
35.0	12.50[†]	8.83[†]

[†] Extrapolated values; [¶] ref 116-118

Table 1.3 Second-order rate constants k_2 for oxidation of ascorbic acid by $[FeL_3]^{3+}$ at 20 °C and I = 1.0 M[¶]

Oxidant	ΔE^{ϕ}/V	k_2/M^{-1} s^{-1}
1) $[Fe(dmphen)_3]^{3+}$	0.97	4.1×10^8
2) $[Fe(mphen)_3]^{3+}$	1.02	7.9×10^8
3) $[Fe(phen)_3]^{3+}$	1.06	1.5×10^9
4) $[Fe(cphen)_3]^{3+}$	1.12	7.5×10^9

[¶] ref 122

Xu and Jordan [125] have made a careful investigation on the ascorbic acid reduction of $[Fe(OH_2)_6]^{3+}$ in dilute aqueous acid, $[H^+]$ = 0.01-0.15 M, at 16 °C. This acidity range was selected in order to avoid oligomerization of aqueous Fe(III) at higher pH. Under the condition of excess of Fe(III), the redox reaction is biphasic with a rapid increase in absorbance at 560 nm followed by a slow decrease. The faster reaction has been assigned to formation of an Fe(III)-ascorbate intermediate complex; Fe(III) was used in excess to ensure

1.2.5 The Redox Chemistry of the Platinum(IV)/(II) Couple

Inorganic platinum chemistry is dominated by four-coordinate Pt(II) and six-coordinate Pt(IV) compounds [134]. Since stable oxidation states of platinum differ by two electrons, chemical and electrochemical reductions of Pt(IV) compounds, such as $[PtX_6]^{2-}$ (X = halide), normally involve transfer of two electrons and loss of two ligands in a reductive elimination process. Conversely, oxidation of Pt(II) with halogens involves a two-electron oxidative addition [135-137].

In principle, monomeric Pt(III) compounds could be expected to be formed as one-electron transfer products in the overall Pt(IV)/Pt(II) redox process. However, monomeric and paramagnetic Pt(III) compounds are rather rare and in many instances they have been detected only through observation of their EPR signals [138,139].

Platinum(IV) compounds have the same low-spin d^6 electronic configuration as the Co(III), Rh(III), and Ir(III) compounds which are known to be characteristically substitution inert [140]. It is not, therefore, surprising that Pt(IV) compounds are inert to ligand exchange reactions. However, these compounds are special in that they undergo Pt(II)-catalyzed substitution which was first postulated by Basolo *et al.* [141]. In the presence of Pt(II) complexes, the reactions are believed to involve a two-electron redox switch between the Pt(II) and Pt(IV) complexes according to the reaction sequence (1.12) - (1.15) [142-144].

$$Pt^{II}L'_4 + X = Pt^{II}L'_4X \text{ , fast} \qquad (1.12)$$

$$\textit{trans}\text{-}[\mathbf{Pt}^{IV}L_4ZY] + Pt^{II}L'_4X = Y\text{-}\mathbf{Pt}^{IV}L_4\text{-}Z\text{-}Pt^{II}L'_4\text{-}X \qquad (1.13)$$

$$Y\text{-}\mathbf{Pt}^{IV}L_4\text{-}Z\text{-}Pt^{II}L'_4\text{-}X = Y\mathbf{Pt}^{II}L_4 + \textit{trans}\text{-}[Pt^{IV}L'_4ZX] \qquad (1.14)$$

$$Y\mathbf{Pt}^{II}L_4 = Y + \mathbf{Pt}^{II}L_4 \text{ , fast} \qquad (1.15)$$

The rate-controlling step for the reaction sequence (1.12) - (1.15) could be reaction (1.13) or (1.14) with a rate constant k [145]. The reaction rate is defined by Eqn. (1.16).

$$\text{Rate} = \frac{kK[X][PtL'_4][PtL_4ZY]}{1 + K[X]} \qquad (1.16)$$

If the concentration of the entering ligand X and/or the formation constant K for the five-coordinate Pt(II) complex, PtL'_4X, is small, then K [X] <<1 and Eqn. (1.16) is reduced to a rate law (Eqn. 1.17) characteristic of Pt(II)-catalyzed Pt(IV) substitution reactions.

$$\text{Rate} = kK\,[X]\,[PtL'_4]\,[\mathbf{Pt}L_4ZY] \qquad (1.17)$$

There are two characteristic features of Pt(II)-catalyzed substitution reactions of Pt(IV); namely, retention of the *trans* geometry for the substitution product PtL'_4ZX and exchange of platinum atoms between the catalyst Pt(II) and substrate Pt(IV) complexes.

Focusing attention on the bridging atom Z, a Pt(II)-catalyzed substitution reaction of Pt(IV) could be regarded as a bimolecular nucleophilic substitution in which the nucleophilic $Pt^{II}L'_4X$ replaces $\mathbf{Pt}^{II}L_4Y$ from the reaction center Z^+ [146]. It can also conveniently be considered as an oxidative addition reaction of the Pt(II) catalyst or a reductive elimination reaction of the substrate Pt(IV) complex.

Apart from the Pt(II)-catalyzed pathway, there are two mechanistic possibilities for reduction of Pt(IV) compounds, depending upon the nature of reducing agent: (i) Outer-sphere one-electron transfer involving a transient Pt(III) intermediate, and (ii) a halide-bridged mechanism involving an attack by the reducing agent on a halide coordinated *trans* to a good leaving group.

The first example of a possible noncomplementary outer-sphere redox process has been found in the reduction of several Pt(IV) complexes by an outer-sphere reductant $[Cr(bipy)_3]^{2+}$ [147]. Reductions of *trans*-$[PtX_4L_2]$ (L = neutral ligand; $X_4 = Cl_4$, Cl_2Br_2, Br_4) by ferrocene in hydroxylic solvents, namely methanol, ethanol, 1-propanol, 2-propanol, 2-methyl-2-propanol, and 1,2-ethanediol [148] and a series of Pt(IV) complexes by V(II) in 0.1 M $LiClO_4$ aqueous medium [149] also occur *via* outer-sphere activated complexes. Ferrocene is expected to favour outer-sphere redox mechanism for it is substitution inert and coordinatively saturated. It reacts with the Pt(IV) complexes quantitatively according to Eqn. (1.18).

$$trans\text{-}[PtX_4L_2] + 2[Fe(C_5H_5)_2] \rightarrow trans\text{-}[PtX_2L_2] + 2[Fe(C_5H_5)_2]^+ + 2X^- \quad (1.18)$$

(L = $AsEt_3$, PPr_3, PEt_3, SEt_2, pip)

The reactivity of these complexes is influenced by both the nature of the ligand L, and solvent used. The reaction rate decreases in the order SEt_2 > pip > PEt_3 > PPr_3 > $AsEt_3$ which is believed to be related to the relative d_π-d_π acceptor and σ-donor ability of the ligands. The reduction reactions are faster in more polar solvents implying separation of charges in the activated complex [148]. In fact, a reaction involving separation of charges in the activated complex is expected to show a higher rate in more polar solvents than in less polar ones [150]. The bromo complexes react 10 - 20 times faster than the chloro complexes in all solvents. This has been ascribed mainly to the lower energy required to deform the bromo complexes in order to make them available for the electron transfer [148].

Reduction of Pt(IV) complexes by V(II) could follow either an outer-sphere or an inner-sphere mechanism since V(II) can act as either a one- or two-electron donor. Outer-sphere electron transfer is proposed for the Pt(IV) complexes given in Table 1.4 based on the facts that V(IV), the oxidation product in the case of two-electron transfer, has not been detected and that the rate constants for reduction of these complexes by V(II) (k_V) and $[Ru(NH_3)_6]^{2+}$ (k_{Ru}), an outer-sphere reductant, display linear correlation [149]. The reaction sequence for the outer-sphere electron transfer is given by Eqns. (1.19) and (1.20). The occurrence of outer-sphere reaction appears to preclude a two-electron transfer. Eqn. (1.21) defines

$$V^{II} + Pt^{IV} \rightarrow V^{III} + Pt^{III} \quad (1.19)$$

$$V^{II} + Pt^{III} \rightarrow V^{III} + Pt^{II}, \text{ fast} \quad (1.20)$$

the correlation between k_V and k_{Ru}. The plot of $\log(k_V)$ against $\log(k_{Ru})$ (Fig.1.9) shows satisfactory linearity and since the slope is close to 1.0, required by the modified Marcus

$$\log k_V = (0.87 \pm 0.02)\log k_{Ru} - (1.70 \pm 0.11) \quad (1.21)$$

approach [151], outer-sphere assignments can be made for both reductants.

Table 1.4 Rate constants for the V(II) and $[Ru(NH_3)_6]^{2+}$ reduction of Pt(IV) complexes in 0.1 M aqueous $LiClO_4$ at 15 °C‡

Oxidant	$k_V/M^{-1}\ s^{-1}$	$k_{Ru}/M^{-1}\ s^{-1}$
1) $[PtCl(NH_3)_5]^{3+}$	0.26	17.7
2) *trans*-$[PtCl_2(NH_3)_4]^{2+}$	15.1	2.14×10^3
3) *trans*-$[PtCl_2(NH_2CH_3)_4]^{2+}$	57	7.5×10^3
4) *mer*-$[PtCl_3(NH_3)_3]^{+}$	71	1.78×10^4
5) $[PtCl_6]^{2-}$	2.14 x 104	7.9×10^6

‡ ref 149

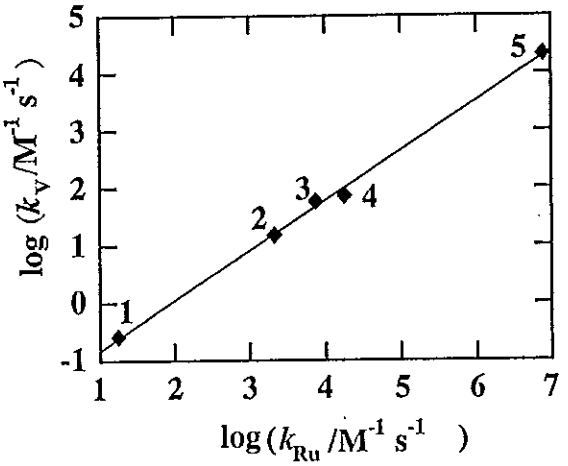


Figure 1.9 Correlation of rate constants for V(II) (k_V) and $[Ru(NH_3)_6]^{2+}$ (k_{Ru}) reductions of platinum(IV) complexes. The numbers refer to the Pt(IV) complexes given in Table 1.4

It is instructive to compare the rate constants for reductions of $[PtCl(NH_3)_5]^{3+}$ and *trans*-$[PtCl_2(NH_3)_4]^{2+}$. The lower rate of reduction of the former might be attributable to the fact that Pt-N bond vibrations *trans* to the chloride are less conducive to electron transfer into the e_g orbital aligned along the axis Cl-Pt^{IV}-NH_3 [152].

Reduction of platinum(IV) complexes of the type *trans*-$[Pt^{IV}X_2L_4]$ (X = Cl, Br; L = neutral or anionic ligand) by anionic and neutral reducing agents takes place through a ligand-bridged activated complex formed by a reductive attack of the reducing agent on the bridging

ligand [153-158] . Reductive-elimination reactions of Pt(IV) complexes following this mechanism could be viewed as consisting of three major steps: (i) formation of a "precursor complex" in which the reactants have been assembled in a solvent cage but are otherwise non-interacting, (ii) movement of the *trans* ligand away from Pt(IV) center and a concomitant formation of a bond between the bridging ligand and the reductant, and (iii) breaking of the bonds between platinum and the leaving ligand and between platinum and bridging ligand and formation of Pt(II) product [155]. Ligand-bridged electron transfer is formally equivalent to transfer of the bridging ligand X to the reductant molecule/ion, as X^+ [140,155,159,160]. Detection of BrCN in the reduction of *trans*-$[PtBr_2(CN)_4]^{2-}$ by CN^- supports the X^+- transfer mechanism [155].

Rates of reduction of Pt(IV) complexes by the atom-transfer mechanism are, as a matter of fact, sensitive to the nature of the bridging atom [160,161]. The nature of the leaving ligand and the four in-plane ligands also influence the rate of reduction. The marked dependence of the rate on the nature of bridging groups is related to the enthalpy of activation, $\Delta H^{\ddagger}$. Favourable $\Delta H^{\ddagger}$ is associated with ligands with good bridging ability . Heavier and more polarizable ligands are effective in bridging while ligands such as NH_3 are not expected to bridge at all [140]. For Pt(IV) complexes with strong-field *trans* ligands such as OH^- and NH_3, the rate of reduction largely depends upon the reducing strength of the anion [160]. Bond-making between reducing anion and the bridging ligand is important for such ligands while it is relatively less so for weak-field ligands such as H_2O.

Both steric and electronic effects of the four in-plane ligands are also important, although these ligands appear to be "non-participating" in the redox process. Bulky ligands are expected to hinder bridge formation while ligands with π-acceptor ability and poor σ-donicity increase the redox reactivity of Pt(IV). The free energy of activation for inner-sphere reductions of Pt(IV) complexes, $\Delta G^{\ddagger}$, is thought to be related to the standard free energy of the redox reaction, $\Delta G^{\emptyset}$, by Eqn. (1.22)

$$\Delta G^{\ddagger} = c\,\Delta G^{\emptyset} + \text{constant} \qquad (1.22)$$

where $\Delta G^{\emptyset} = -nFE^{\emptyset}_{(Pt(IV)/Pt(II))} + \text{constant}$ [162]. The reduction potential of a complex is generally thought to increase linearly with the electron affinity of the central metal atom [163] which, in turn, is postulated to parallel the decreasing contribution from the ligands to the electron density on the metal [164]. The gradient, c, in Eqn. (1.22) varies with the nature of the Pt(IV) complex from *ca.* 0.5 up to 1, which suggests that the transition state bears a close resemblance to the products, *i.e.*, there is a considerable degree of bond making and breaking in the transition state. Pt(IV) complexes containing ligands with π-acceptor properties such as CN^- have relatively high reduction potentials and hence are reduced faster than the ammine analoges.

1.2.6 Activation Parameters and Reaction Mechanism

Although constant temperature is maintained in a given set of experiments, the variation of temperature provides further information about the reaction mechanism. The rate constant-temperature data provide values for the activation parameters, *i.e.*, enthalpy of activation $\Delta H^{\ddagger}$ and entropy of activation $\Delta S^{\ddagger}$. The values of $\Delta H^{\ddagger}$ relate most simply to the differences in bond enthalpies between the transition state and the reactants. But differences in the solvation energies of the reactants and transition state, particularly for species bearing ionic charges, could be significant and should be considered. $\Delta S^{\ddagger}$, having a more direct connection with the molecularity, structure, and charge of the activated complex, is regarded as more useful in providing insight into reaction mechanism [165].

Kinetic data collected at different pressures yield the volume of activation $\Delta V^{\ddagger}$ for pressure-dependent reactions. There is often a strong correlation between volume of activation and entropy of activation. The increase in volume resulting from bond stretching on forming a dissociative transition state is generally accompanied by an increase in entropy from the greater freedom of the leaving group. The use of $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$ in the diagnosis of reaction mechanism is reliable if there are no strong substrate-solvent interactions. Solvation

changes consequent on transition-state formation may make a significant contribution to the experimentally obtained values for $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$.

The Eyring's equation, Eqn. (1.23), is commonly used to express the rate constant of elementary reactions as a function of temperature. In this equation k denotes the rate constant,

$$\ln(k/T) = \ln(K_B/h) + \Delta S^{\ddagger}/R - \Delta H^{\ddagger}/RT \qquad (1.23)$$

R the universal gas constant (8.314 J K^{-1} mol^{-1}), K_B Boltzmann's constant (1.38 x 10^{-23} J K^{-1}), h planck's constant (6.626 x 10^{-34} J s), and T the absolute temperature. Only those rate constants whose concentration dependencies are resolved can be treated by the Eyring's equation. Meaningful values for the activation parameters cannot be obtained if pseudo-order rate constants are correlated.

The activation enthalpy of a very rapid reaction is normally quite small [166] but can also be high if the value of $\Delta S^{\ddagger}$ is more positive so as to compensate. The entropy of activation associated with a second-order reaction that occurs by a bimolecular step is large and negative. In ionic reactions, $\Delta S^{\ddagger}$ is largely charge-controlled where the reaction between unlike charged reactants is often attended by a positive entropy of activation because the solvent molecules are less restricted around an activated complex of reduced charge and thus are released from it. Reactions between reactants with the same sign of charge are accompanied by a negative entropy of activation since a transition state of increased charge is formed.

2.0 Experimental

2.1 Chemicals

The compounds *trans*-[$PtCl_4(NH_3)(Tz)$] (**1**), *trans*-[$PtCl_4$(cha)(NH_3)] (**2**), *cis*-[$PtCl_4$(cha)(NH_3)] (**3**), and *cis*-[$PtCl_4(NH_3)_2$] (**4**) were kindly donated by Dr. Nicholas Farrell (Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia, USA). Compounds *trans, trans, trans*-[$PtCl_2(OH)_2$(cha)(NH_3)] (JM335) (**5**), *cis, trans, cis*-[$PtCl_2(OH)_2$(cha)(NH_3)] (JM149) (**6**), *cis, trans, cis*-[$PtCl_2(OAc)_2$(cha)(NH_3)] (JM216) (**7**), *cis, trans, cis*-[$PtCl_2(OCOC_3H_7)_2$(cha)(NH_3)] (JM221) (**8**), *trans, cis, cis* - [$PtCl_2(OAc)_2$(cha)(NH_3)] (JM394) (**9**), and *trans, trans, trans*-[$PtCl_2(OAc)_2$(cha)(NH_3)] (JM576) (**10**) were kindly supplied as a loan by the Johnson Matthey Technology Center (Reading, Berkshire, UK). Compounds *trans*-[$PtCl_4(NH_3)_2$] (**11**) (Strem Chemicals), $K_2[PtCl_6]$ (**12**) (Alfa Products), and $K_2[PtBr_6]$ (**13**) (Alfa Products) were used as received. The compounds *trans*-[$PtCl_2(en)_2]Cl_2$ (**14**), *trans*-[$PtBr_2(NH_3)_4]Br_2$ (**15**) and $[Pt(NH_3)_6]Cl_4$ (**16**) were prepared by adopting literature methods [145,167,168].

Glutathione (Merck, ≥ 99%), L-cysteine (ICN Biomedicals Inc.), 2-mercaptopropanoic acid (Acros, 95%, ρ 1.19 kg/L), DL-homocysteine (Sigma), DL-penicillamine (Janssen, >99%), DL-mercaptosuccinic acid (Acros, 99%), N-acetyl-L-cysteine (Janssen, 98%), L-ascorbic acid (Merck, pa > 99.7%), sodium hydrogen ascorbate (ICN Biochemicals Inc.), $CH_2ClCOOH$ (Acros, pa), CH_3COOH, glacial (Merck, 100%), CH_3COONa (Merck, pa, ≥ 99%), $Na_2HPO_4.2H_2O$ (Merck, pa, ≥ 99.5%), $NaH_2PO_4.H_2O$ (Merck, pa, ≥ 99%), $Na_3PO4.12H_2O$ (Merck), oxidized glutathione (Acros), tris(hydroxymethyl)aminomethane, TRIS (Merck, pa), $NaHCO_3$ (Merck, pa), Na_2CO_3 (Merck, pa), $HClO_4$ (Merck, pa, 70-72%), $NaClO_4$, anhydrous (ACROS, 99%), D_2O (ACROS, 99.8% D), NaCl (Merck, pa, ≥ 99.5%), NaBr (FisherChemical), EDTA $C_{10}H_{14}O_8N_2Na_2.2H_2O$ (Chemtam, pa), and $Cu(NO_3)_2.3H_2O$ (Riedel-De Haen AG) were used as received. Other chemicals used were of analytical grade.

2.2 Instrumentation

UV/VIS spectra were recorded with Milton Roy 3000 diode array and Cary 300 Bio UV/VIS spectrophotometers using 1.00 cm Suprasil cells. Kinetic measurements were made using an Applied Photophysics Bio Sequential SX-17MX stopped-flow ASVD and the Cary 300 spectrophotometers. Constant temperature (± 0.1 °C) was maintained by an external RM6 LAUDA circulating-water bath for stopped-flow measurements and a Cary peltier thermostat (± 0.02 °C) coupled with a circulating-water temperature control unit for measurements using the Cary 300 spectrophotometer. A Metrohm 632 and an Orion Research Expandable ion-Analyzer, model EA 920, digital pH meters coupled with a Metrohm and an Orion combined glass electrodes were used to measure pH. Standard buffers of pH 2, 4, 7, and 10, obtained from Merck, were used to calibrate the electrodes. Activities of oxonium ions OH_3^+, $a_H = 10^{-pH}$, were calculated directly from pH meter readings. ^1H and ^{195}Pt-NMR spectra were recorded with a Varian Unity 300 spectrometer operating at 299.779 and 64.279 MHz, respectively. Those platinum(IV) compounds with poor water solubility were dissolved by sonication using a Bransonic 220 ultrasonic water bath.

2.3 Preparation of compounds 14 - 16

The compounds *trans*-$[PtCl_2(en)_2]Cl_2$ **(14)** and *trans*-$[PtBr_2(NH_3)_4]Br_2$ **(15)** were prepared by oxidizing $[Pt(en)_2]Cl_2$ with 30 % hydrogen peroxide in *ca.* 6 M hydrochloric acid medium and by oxidizing $[Pt(NH_3)_4]Cl_2$ with five fold excess of bromine in 1.0 M hydrobromic acid medium, respectively. The products were recrystallized from hydrochloric and hydrobromic acids as appropriate.

$[Pt(NH_3)_6]Cl_4$ (16). This compound was prepared starting from $[Pt(NH_3)_4]Cl_2$ by adopting the literature method [167]. 1.6 g (4.79 mmol) $[Pt(NH_3)_4]Cl_2$ was dissolved in 25 mL of water and equal volume of conc. HCl and 2 mL of 30 (vol. %) hydrogen peroxide was added after heating the solution to 40 °C. The warm solution was cooled to room temperature

and then to 4 °C in the refrigerator. The product, *trans*-$[PtCl_2(NH_3)_4]Cl_2$, was filtered and washed with ethanol and acetone and then dried in air. Yield 1.48 g (3.65 mmol).

To 1.0 g (2.47 mmol) *trans*-$[PtCl_2(NH_3)_4]Cl_2$ dissolved in 150 mL water at 90 °C was added 4.0 g (30.3 mmol) $(NH_4)_2SO_4$ and 10 mL concentrated ammonia. More water and concentrated ammonia was added from time to time keeping the temperature at 90 – 95 °C and with stirring for about 1h. The hot solution was cooled to 60 °C, filtered, washed with small portions of ethanol and ether, and dried at 60 – 80 °C. The mother liquor was heated to boiling and the remaining 0.48 g (1.2 mmol) *trans*-$[PtCl_2(NH_3)_4]Cl_2$ (in small portions) and 10 mL concentrated ammonia was added. The product $[Pt(NH_3)_6](SO_4)_2$ obtained from both preparations was combined. Yield 0.78 g (1.6 mmol).

0.78 g $[Pt(NH_3)_6](SO_4)_2$ was suspended in 60 mL water and about 20 mL of 1.0 M NaOH was added dropwise in order to dissolve the compound completely. The resulting solution was heated to 90 °C and 20 mL of 64 mM $BaCl_2.2H_2O$ was added dropwise. The solution was then digested for *ca.* 30 min, cooled to room temperature and the precipitated $BaSO_4$ was removed by filtration. 10 mL concentrated hydrochloric acid was added to the filtrate and the volume was decreased to about 30 mL by evaporation. The desired product, $[Pt(NH_3)_6]Cl_4$, was filtered and washed with ethanol. Yield 0.565 g (1.29 mmol).

2.4 Solutions

The following buffers were used to maintain constant pH in the regions indicated: chloroacetic acid/sodium chloroacetate (2.00 - 3.30), acetic acid/sodium acetate (3.50 - 5.50), sodium phophate/disodium phosphate (5.70 - 7.00), TRIS-HCl or -$HClO_4$ (7.00 - 9.00), hydrogen carbonate/carbonate (9.00-10.00), and disodium phosphate/phosphate (10.00 - 11.22). All buffers were deoxygenated by flushing with argon for about 30 min. Fresh stock solutions (10 - 50 mM) of the thiols and ascorbic acid (10 – 100 mM) prepared in buffer were used for each kinetic measurement. The pH of these solutions was checked and adjusted, when different from desired values, by adding few drops of strong base. Stock solutions (0.5

– 1.0 mM) of the Pt(IV) compounds **2**, **3**, and **4** were prepared in aqueous sodium chloride solutions. Several hours of sonication was required to dissolve compound **2** to a concentration of *ca.* 1 mM. The stock solutions of the platinum(IV) compounds were stable to hydrolysis and could be stored in the darkness for about a week without observable changes. Measuring solutions of these compounds were prepared by diluting 0.5 -1.5 mL of stock solution with buffer to 10 mL and those of compound **1** by dissolving 0.5 – 1.0 mg solid sample in water (≤ 1 mL) and then diluting with buffer to 10 mL. Weighed samples of compounds **5** and **11** - **14** were directly dissolved in buffer and used for kinetic measurements. Compounds **7** (JM216) and **8** (JM221) were dissolved in water since they tend to hydrolyze when dissolved directly in buffer . A few minutes of sonication was sufficient to dissolve these compounds to a concentration of 0.5 mM. Solutions of compounds **9** (JM394) and **10** (JM576) were prepared by dissolving weighed samples (1.5 – 5 mg) in small volume of water and then diluting with buffer containing 10 - 20 mM chloride to 10 mL. Water was doubly distilled from quartz.

2.5 Kinetic Measurements

2.5.1 General Measuring Conditions

The redox reactions were investigated in aqueous medium of ionic strength 1.0 M adjusted with $NaClO_4$ using stopped-flow or conventional UV/VIS spectrophotometry . Fast reactions were studied at 25 °C using the Applied Photophysics stopped-flow and the slower ones at 35 °C using the Cary 300 spectrophotometers. Kinetic measurements for the chloro- and bromoplatinum(IV) compounds were made in the presence of free chloride or bromide as appropriate in order to suppress hydrolysis of the compounds. Na_2H_2edta (0.5 – 3 mM) was present in all buffers to avoid autoxidation of the thiols and ascorbic acid [115,169-173]. Na_2H_2edta is known to sequester paramagnetic metal ions such as Fe(III) and Cu(II) which might catalyze oxidation of thiols and ascorbic acid by dioxygen [172]. All kinetic measurements were made under pseudo-first-order conditions of at least a 10-fold excess of

the thiols and ascorbic acid by monitoring absorbance decrease at wavelengths where the reductant molecule/ion and products do not absorb appreciably. Observed pseudo-first-order rate constants k_{obsd} were obtained as average values from three to six repetitive measurements.

A very practical advantage of pseudo-first-order kinetics is that the pseudo-first-order rate constant k_{obsd} is independent of the initial concentration of the reactant whose concentration is being monitored, *i.e.*, the deficient reagent. This means that collection of absorbance-time data can be started at any time arbitrarily defined as $t = 0$.

2.5.2 Kinetic Measurements for Reduction of Platinum(IV) Anticancer Active Compounds by Thiols

Reductions of the anticancer active tetrachloroplatinum(IV) compounds, *trans*-$[PtCl_4(NH_3)(Tz)]$ (**1**), *trans*-$[PtCl_4(cha)(NH_3)]$ (**2**), *cis*-$[PtCl_4(cha)(NH_3)]$ (**3**), and *cis*-$[PtCl_4(NH_3)_2]$ (**4**) by glutathione and of *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**) by L-cysteine, 2-mercaptopropanoic acid, DL-homocysteine, DL-penicillamine, DL-mercaptosuccinic acid, glutathione, and N-acetyl-L-cysteine have been investigated in the pH regions 2.0 - 7.0 and 6.80 - 11.22, respectively, using the stopped-flow technique. Kinetic measurements for compounds **1 - 4** were made in the presence of 0.5 - 2.0 mM Na_2H_2edta and 0.2 –0.9 M chloride. Similarly, reduction of **5** (JM335) by the thiols was investigated in the presence of 100 mM chloride and 2 – 3 mM Na_2H_2edta.

Single-exponential kinetic traces were obtained in all cases and the observed pseudo-first-order rate constants k_{obsd} were calculated by an on-line nonlinear least-squares analysis of the absorbance-time data using an Applied Photophysics software package [174]. The effect of free chloride on the pseudo-first-order rate constants was studied for compounds **3** and **4** at constant pH, ionic strength, and total concentration of glutathione. Results of this experiment are presented in Table 3.3. The dependence of k_{obsd} on the initial concentrations of Pt(IV) was studied for compound **4** (*cf.* Table 3.1).

and that at *ca.* 2.06 ppm in spectrum (a) corresponds to the acetates coordinated to the product platinum(II) complex. Compound **8** (Page 2) is assumed to be reduced to the same product as

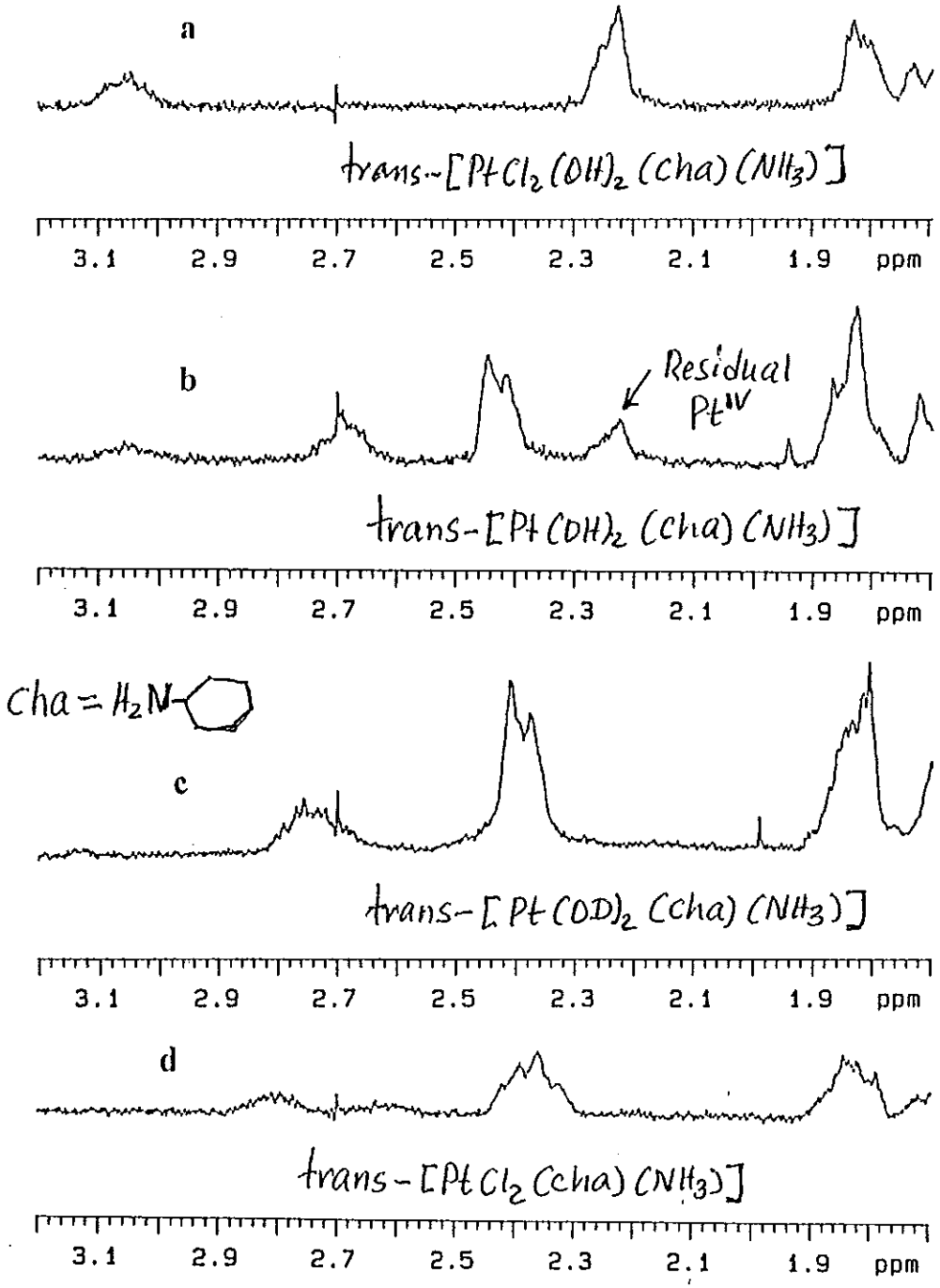


Figure 3.2 Proton NMR spectra of: (a) *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**), (b) the product for reduction of **5** (slight excess) by penicillamine at pH ~ 9, (c) *trans*-$[Pt(OD)_2(cha)(NH_3)]$, (d) and *trans*-$[PtCl_2(cha)(NH_3)]$ (JM334), all recorded at 25 °C

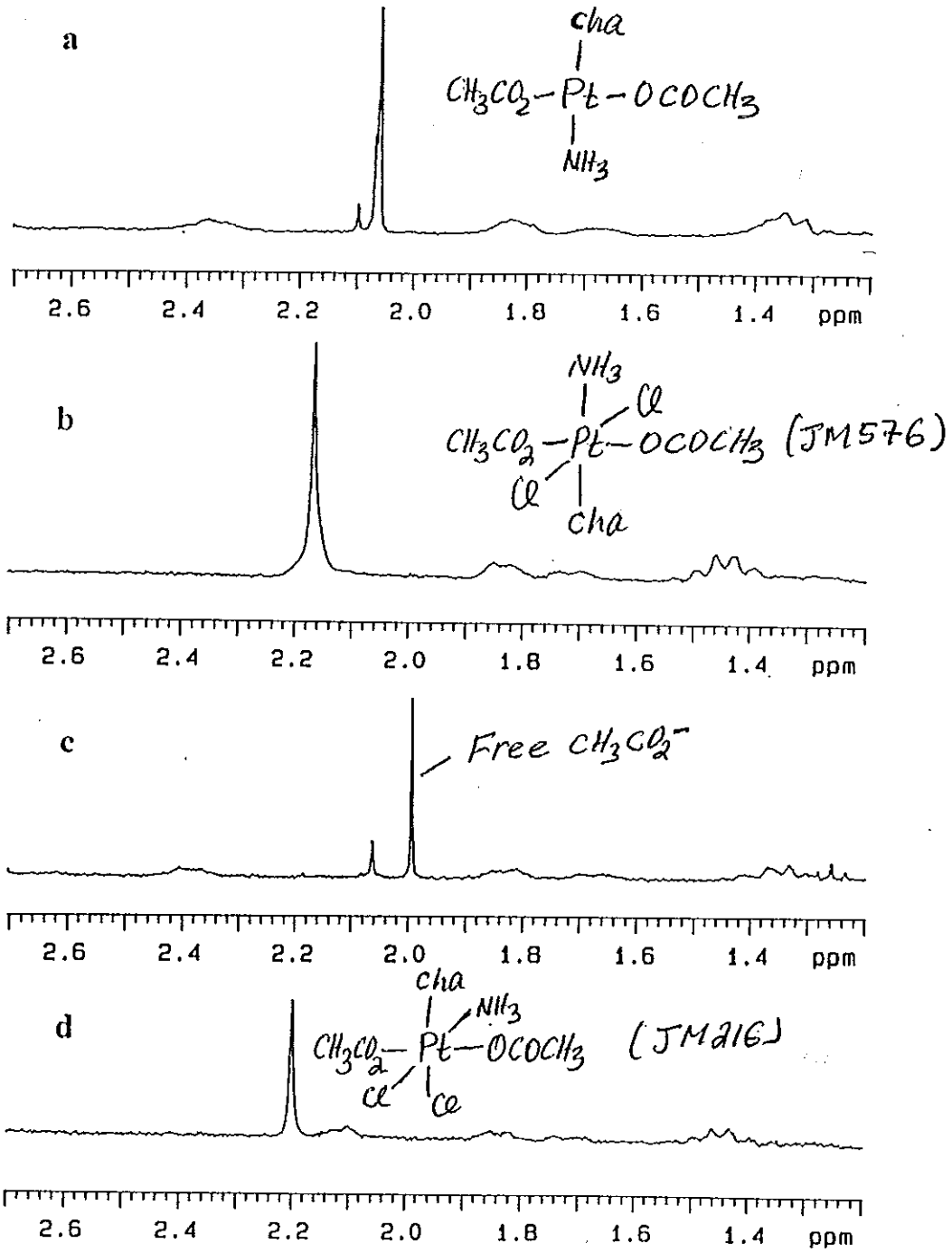


Figure 3.3 Proton NMR spectra of: (a) the product for reduction of *trans, trans, trans*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**10**) by ascorbate in a 1:1 molar ratio, (b) **10**, (c) the product for reduction of *cis, trans, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**7**) by ascorbate (excess), and (d) **7**

that of compound **7**. Compound **9** (Page 3) is reduced to *cis*-$[Pt(OAc)_2(cha)(NH_3)]$. Ascorbic acid is known to be oxidized to dehydroascorbic acid (DHA) both by one- and two-electron oxidants [120,121,182-184].

Unfortunately, a spectrophotometric study of the reduction of $[Pt(NH_3)_6]^{4+}$ **(16)** by ascorbate was not feasible for lack of a suitable wavelength for monitoring the reaction. The UV-vis absorption spectra of **16** and the reduction product $[Pt(NH_3)_4]^{2+}$ are shown in Fig. 3.4.

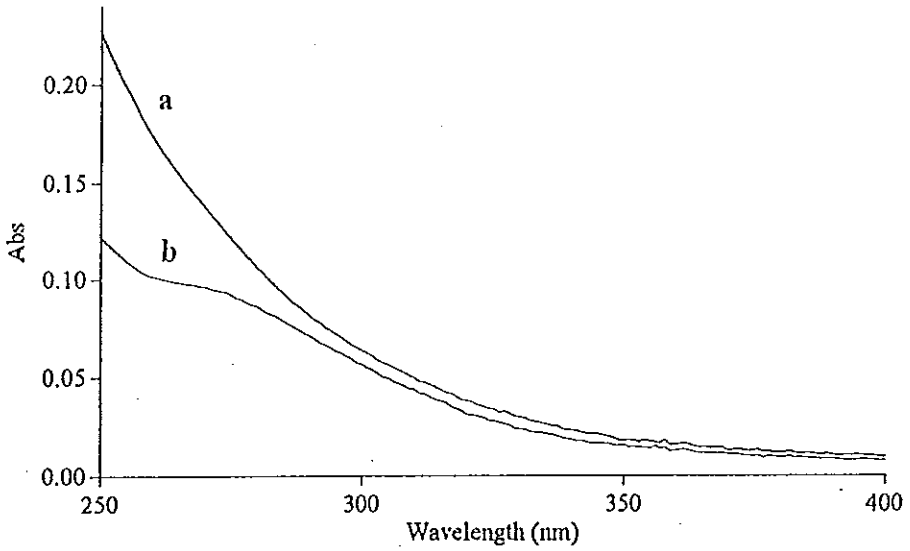


Figure 3.4 UV-vis absorption spectra of $[Pt(NH_3)_6]^{4+}$ (a) and $[Pt(NH_3)_4]^{2+}$ (b)

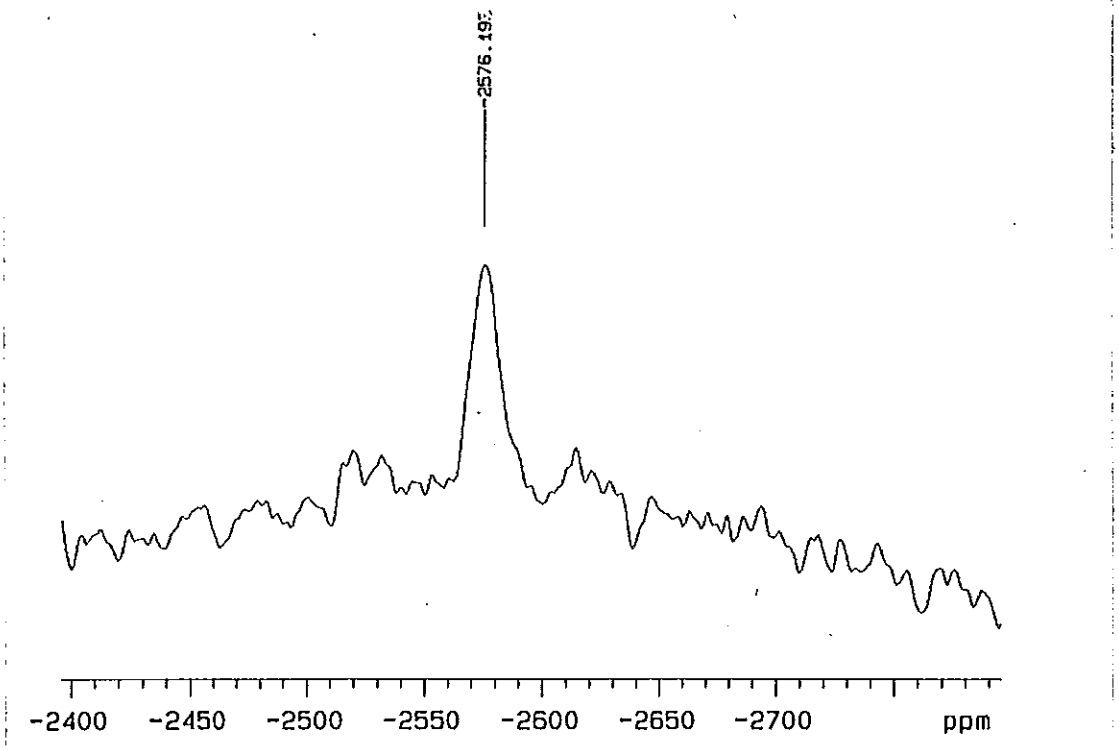


Figure 3.5 ^{195}Pt NMR spectrum of the product for reduction of $[Pt(NH_3)_6]^{4+}$ by ascorbic acid at 35 °C and pH 6.

Reduction of **16** to $[Pt(NH_3)_4]^{2+}$ has been confirmed by ^{195}Pt NMR measurements (Fig. 3.5). Ascorbate reduction of **16** is necessarily an outer-sphere reaction since the ammonia ligands are non-bridging.

3.2 Stoichiometry

The stoichiometry [Pt(IV)] : $[RSH]_{tot}$ for reduction of *trans*-$[PtCl_2(CN)_4]^{2-}$ by a series of thiols has been established to be 1:2 [157]. A spectrophotometric study of the present systems was not feasible because of interference from slow subsequent substitution processes. Cysteine, glutathione, and amino acids containing a thiol group are known to react readily with *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ [180,181]. The reaction conditions chosen here, *i.e.* excess of thiol, suggest that a slow replacement of chloride by RSH or RS^- is possible at the platinum(II) product compounds. By analogy, reduction of the anticancer active compounds **1** – **4** and **5** by the thiols is assumed to follow the same stoichiometry of Pt(IV):RSH = 1:2. Glutathione is observed to be oxidized quantitatively by compounds **4** and **5** in a 1:2 Pt(IV):GSH reaction mixture confirming the assumed 1:2 stoichiometry (*cf.* Fig. 3.1).

Interestingly, the stoichiometries for reduction of *trans*-$[PtX_2(CN)_4]^{2-}$ (X = Cl, Br) by SCN^-, $S_2O_3^{2-}$, and SO_3^{2-} are reported to be 3:1, 1:2, and 1:1 ([Pt(IV)]:[Red]), respectively [155]. These inorganic anions reduce the halo-platinum(IV) compounds by the same intimate mechanism despite the difference in their reaction stoichiometry. On the basis of the mole ratio $[Pt(IV)]/[SCN^-] = 3.0$, and detection of SO_4^{2-} by precipitation of $BaSO_4$, reduction of *trans*-$[PtCl_2(CN)_4]^{2-}$ and $[PtBr_2(CN)_4]^{2-}$ by SCN^- is formulated as

$$[PtX_2(CN)_4]^{2-} + SCN^- \rightarrow [Pt(CN)_4]^{2-} + XSCN + X^- \qquad (3.1)$$

$$2[PtX_2(CN)_4]^{2-} + XSCN + 4H_2O \rightarrow$$

$$2[Pt(CN)_4]^{2-} + SO_4^{2-} + HCN + 5X^- + 7H^+ \qquad (3.2)$$

Ascorbic acid is a well-known two-electron reductant whose reactions with platinum(IV) compounds are expected to follow a 1:1 stoichiometry. This has been demonstrated by a spectrophotometric titration of a fixed concentration of $[PtBr_6]^{2-}$ by

ascorbic acid at 370 nm. The break in the plot of absorbance *versus* the mole ratio $[H_2Asc]_{tot}/[Pt(IV)]$ occurred at the expected ratio of 1:1 (Fig. 3.6).

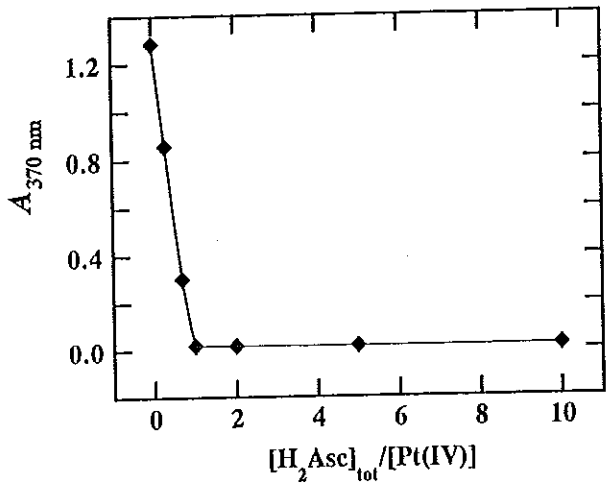


Figure 3.6 Stoichiometric plot for ascorbic acid reduction of $[PtBr_6]^{2-}$ (**13**). Conditions: $[Pt(IV)] = 2 \times 10^{-4}$ M, pH = 6.49, $[Br^-] = 0.7$ M, $[Na_2H_2edta] = 3$ mM, $I = 1.0$ M

3.3 Kinetics

Single-exponential kinetic traces were obtained in all cases indicating that the redox reactions are first-order in the platinum(IV) complexes. The pseudo-first-order rate constants k_{obsd} are not dependent on the initial concentrations of the platinum(IV) complexes confirming that the reactions are first-order in the platinum(IV) complexes (Table 3.1 and 3.2).

Plots of k_{obsd} *vs.* $[Red]_{tot}$ (Red = thiol, ascorbic acid) are linear with zero intercept for reactions investigated in the presence of Na_2H_2edta. The plots of k_{obsd} *vs.* $[H_2Asc]_{tot}$ obtained for reduction of compounds **11** – **14** at pH 5.75 are illustrated in Fig. 3.7 . Small intercepts were observed for reactions studied under aerobic conditions in the absence of Na_2H_2edta. Such intercepts are caused by autoxidation of the thiols and ascorbic acid catalyzed by transition metal ions such as Cu^{2+} and Fe^{3+}. Addition of Cu^{2+} to reaction mixtures has the effect of increasing the intercepts, and addition of Na_2H_2edta removes them (Figs. 3.8 and 3.9).

subsequent rapid step (*vide infra*). Since there are no proton-related equilibria associated with the platinum(IV) compounds, the pH dependence of k is attributed to displacement of protolytic equilibria involving the various species of the thiols and ascorbic acid. These

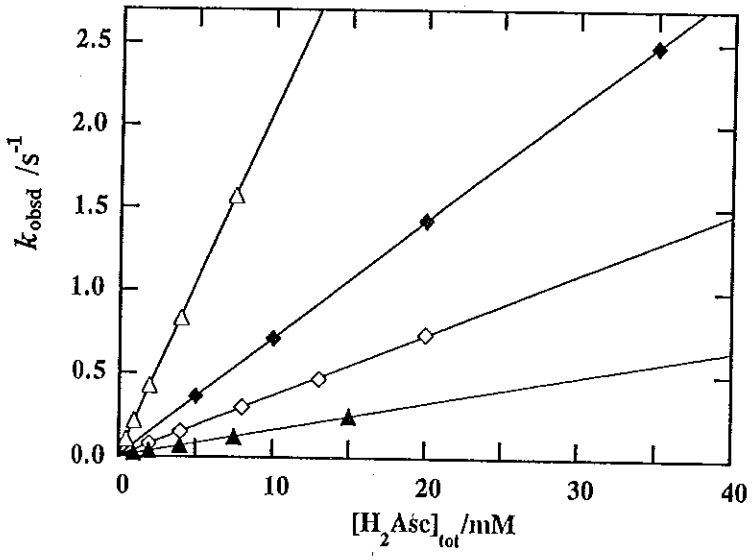


Figure 3.7 Plots of k_{obsd} *vs.* $[H_2Asc]_{tot}$ for $[PtBr_6]^{2-}$ (Δ), *trans*-$[PtCl_2(en)_2]^{2+}$ (♦), *trans*-$[PtCl_4(NH_3)_2]$ (◇), and $[PtCl_6]^{2-}$ (▲). Reaction conditions: pH 5.74, 25 °C, 3 mM EDTA, $[Cl^-]$ = 0.7 M (♦,◇,▲), and $[Br^-]$ = 0.7 M (Δ)

Table 3.3 Effect of added chloride on the pseudo-first-order rate constants for reductions of compounds **3** and **4** by glutathione at 25 °C and pH 4.5

	$^{\dagger}k_{obsd}$/s^{-1}	
$[Cl^-]$/M	(a) *cis*-$[PtCl_4(cha)(NH_3)]$ (**3**)	(b) *cis*-$[PtCl_4(NH_3)_2]$ (**4**)
0.2	0.254 ± 0.004	0.150 ± 0.001
0.3	0.247 ± 0.001	0.154 ± 0.002
0.4	0.252 ± 0.006	0.146 ± 0.001
0.5	0.259 ± 0.002	0.149 ± 0.001

† Conditions: (a) $[GSH]_{tot}$ = 6 mM, [Pt(IV)] = 5 x 10^{-5} M; (b) $[GSH]_{tot}$ = 5 mM GSH, [Pt(IV)] = 8 x 10^{-5} M

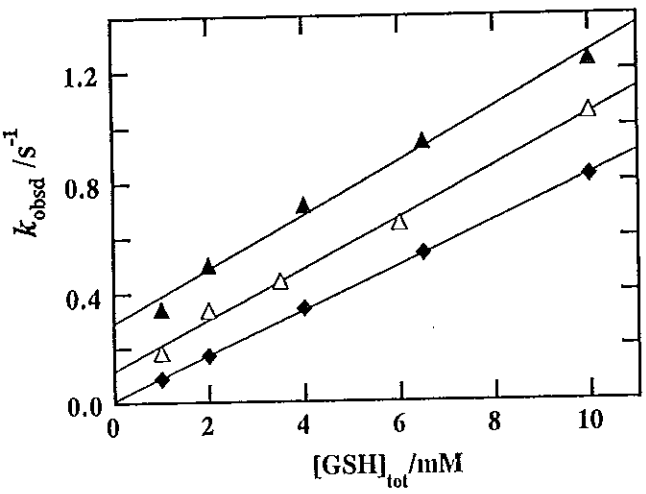


Figure 3.8 Effect of added Cu^{2+} on the pseudo-first-order rate constants k_{obsd} for reduction of *cis*-$[PtCl_4(NH_3)_2]$ (**4**) by glutathione at pH 5.04, 25 °C, and I = 1.0 M. Conditions: $[Cu^{2+}]$ = 1 x 10^{-5} M, aerobic (▲); No added Cu^{2+}, aerobic (Δ); $[Cu^{2+}]$ = 1 x 10^{-5} M, [EDTA] = 3 mM, anaerobic (♦); [Pt(IV)] = 1 x 10^{-4} M (▲, Δ,♦)

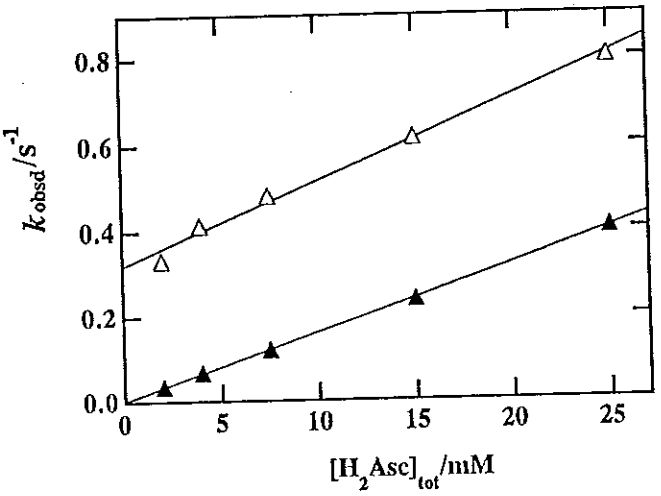
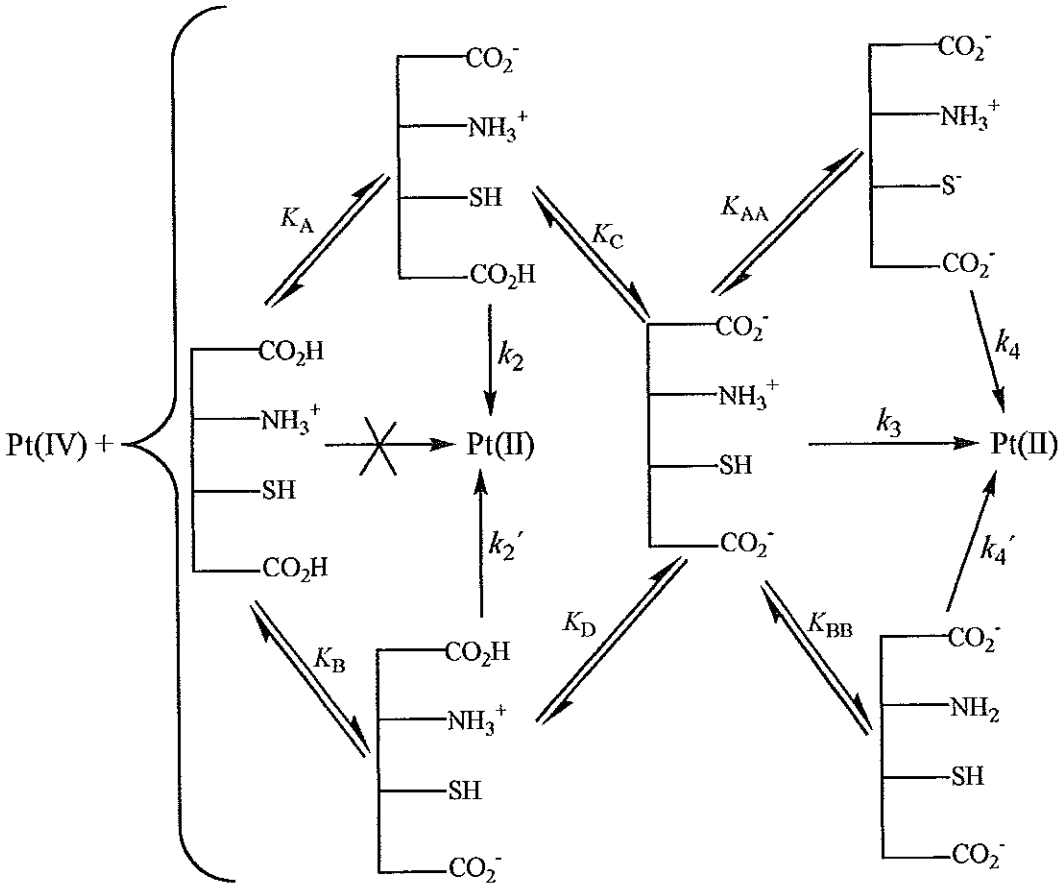


Figure 3.9 Effect of added Cu^{2+} on the pseudo-first-order rate constants for reduction of $[PtCl_6]^{2-}$ (**12**) by ascorbic acid at pH 5.80, 25 °C, and I = 1.0 M. Conditions: $[Cu^{2+}]$ = 1 x 10^{-5} M, aerobic (Δ); $[Cu^{2+}]$ = 1 x 10^{-5} M, [EDTA] = 3 mM, anaerobic (▲); [Pt(IV)] = 1 x 10^{-4} M (Δ, ▲)



Scheme 3.1 Reaction model for reduction of the tetrachloroplatinum(IV) anticancer active compounds **1** - **4** by glutathione.

$$k = \frac{(k_2K_A + k_2'K_B)a_H^{\,2} + k_3K_AK_Ca_H + k_4K_AK_CK_{AA}}{a_H^{\,3} + (K_A + K_B)a_H^{\,2} + K_AK_Ca_H + K_AK_C(K_{AA} + K_{BB})} \qquad (3.5)$$

In deriving this equation, the contribution of the k_4'- pathway in Scheme 3.1 to the overall reduction of **1** is assumed to be insignificant considering the large difference between the reactivities of RSH and RS$^-$ [157]. This equation was fitted to the experimental data by a weighted nonlinear least-squares analysis with a_H taken as an independent variable; (k_2K_A + $k_2'K_B$), k_3, and k_4 as adjustable; K_A, K_B, K_C, K_{AA}, and K_{BB} as constant parameters. The values

for these parameters and for those of the other thiols taken from literature are collected in Table 3.4.

The value for $k_2K_A + k_2'K_B$ obtained from the curve fitting is (4.02 ± 0.04) x 10^{-2} s^{-1} whereas those for k_2 and k_2'cannot be obtained directly. However, since K_A is more than ten times larger than K_B, the sum of k_2K_A and $k_2'K_B$ can be approximated to $k_2K_A \approx 4.02 \times 10^{-2}$ s^{-1}. The rate constant k_2 can thus be estimated to be 6 M^{-1} s^{-1}.

Eqn. (3.5) does not fit to the data collected for compounds **2** – **4**. Very satisfactory curve fittings are obtained when Eqn. (3.6), which is derived considering GS^- as the predominant reductant, is used. The rate constants for reduction of **2** – **4** by the protonated species of glutathione are indeterminate under the reaction conditions used.

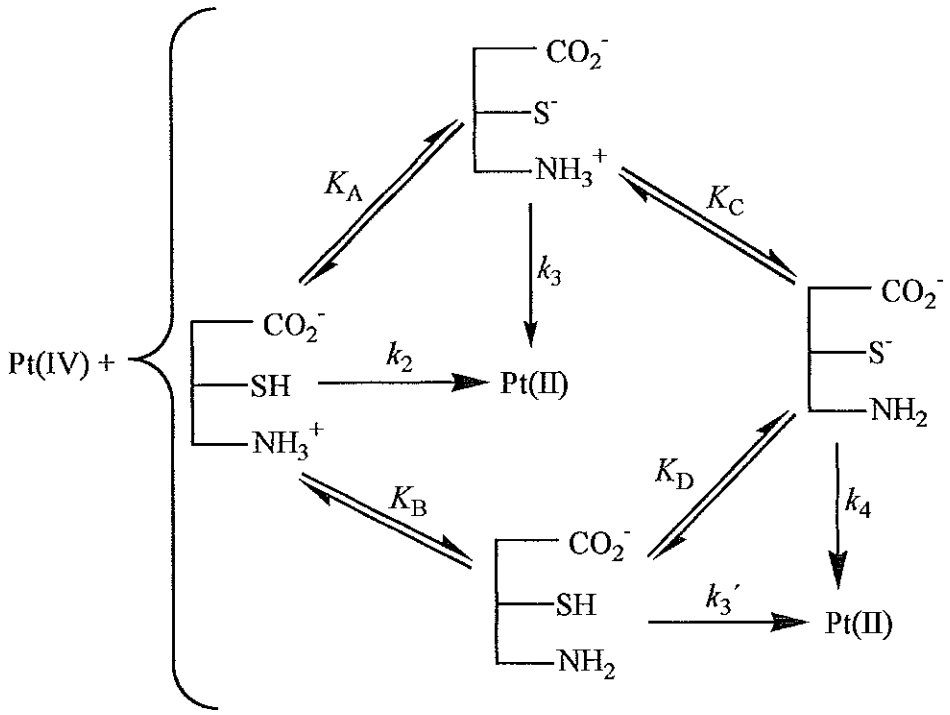
$$k = \frac{k_4 K_{AA} K_A K_C}{(K_A + K_B)a_H^{\,2} + K_A K_C a_H + (K_{AA} + K_{BB})K_A K_C} \qquad (3.6)$$

Reaction models for the reduction of *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**) by the thiols are formulated considering only the species whose concentrations are significant in the pH region 6.80 - 11.22. Eqn. (3.7), derived from Scheme 3.2, was fitted to the experimental data obtained for reductions by cysteine, penicillamine, and homocysteine.

$$k = \frac{k_2 a_H^{\,2} + k_3 K_A a_H + k_4 K_A K_C}{a_H^{\,2} + (K_A + K_B)a_H + K_A K_C} \qquad (3.7)$$

In deriving the preceding equation, the contribution of the k_3'-pathway in Scheme 3.2 to the overall reduction of **5** is assumed to be insignificant for the same reason given in deriving Eqn. (3.5).

Eqn. (3.8) was derived from Scheme 3.3 and fitted to the experimental data collected for reduction of **5** by the diprotic thiols N-acetyl-L-cysteine and 2-mercaptopropanoic acid. A reaction model for reduction of **5** by the triprotic thiol, DL-mercaptosuccinic acid is



Scheme 3.2 Reaction model for reduction of *t,t,t*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**) by L-cysteine, DL-homocysteine, and DL-penicillamine

$$k = \frac{k_2 a_H + k_3 K_{a2}}{a_H + K_{a2}} \tag{3.8}$$

given in Scheme 3.4. Eqn. (3.9) is derived from this scheme and fitted to experimental data.

$$k = \frac{k_3 K_{a2} a_H + k_4 K_{a2} K_{a3}}{a_H^{\,2} + K_{a2} a_H + K_{a2} K_{a3}} \tag{3.9}$$

Eqn. (3.10) was fitted to the data obtained for reduction of **5** by glutathione. In deriving this equation the k_4'-pathway in Scheme 3.5 is neglected since the thiolate species GS^- is much more reactive than the thiol GSH.

$$k = \frac{k_3 a_H^{\,2} + k_4 K_{AA} a_H + k_5 K_{AA} K_{CC}}{a_H^{\,2} + (K_{AA} + K_{BB}) a_H + K_{AA} K_{CC}} \tag{3.10}$$

Table 3.4 Microscopic and macroscopic acid dissociation constants of thiols at 25 °C

Thiol	¶p*K*A	pK_B	pK_C	pK_D	pK_{AA}	pK_{BB}	pK_{CC}	pK_{DD}	Ref.
Gluthione	2.19	3.22	3.45	2.42	8.97	9.17	9.35	9.08	[186]
L-Cysteine	8.40	8.85	10.05	9.60					[179,187]
DL-Homocysteine	9.02‡	9.04‡	9.71‡	9.69‡					[188]
DL-Penicillamine	8.05	8.61	10.29	9.70					[179,187]
N-Acetyl-L-cysteine	pK_{a1} (COOH) = 3.21			pK_{a2} (SH) = 9.55					[179]
2-Mercaptopropionic acid	pK_{a1} (COOH) = 3.38			pK_{a2} (SH) = 9.93					[189]
DL-Mercaptosuccinic acid	pK_{a1} (COOH) = 4.63			pK_{a2} (SH) = 10.26					[187]

¶ The acid dissociation constants refer to the dissociation equilibria shown in Schemes 3.1 – 3.5

‡ 30 °C

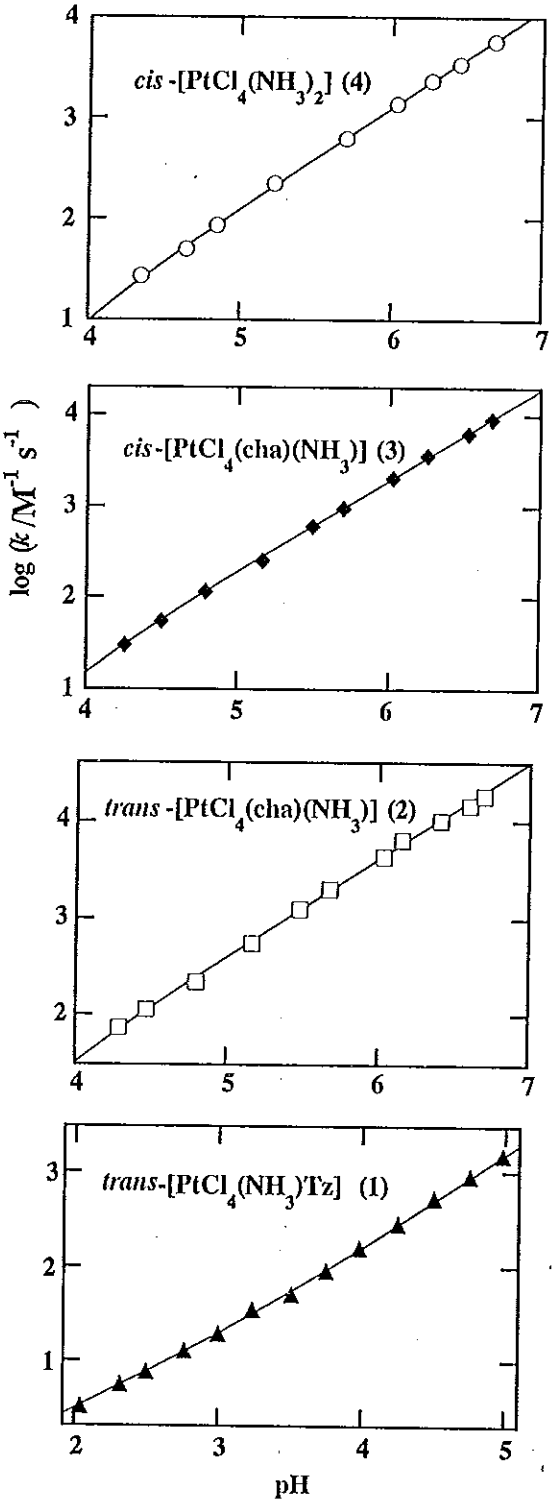


Figure 3.10 Plots of second-order rate constants k as a function of pH for reduction of compounds **1** - **4** by glutathione at 25 °C and I = 1.0 M. The solid lines represent the fits of Eqns. (3.5) to the experimental data obtained for *trans*-$[PtCl_4(NH_3)Tz]$ (**1**) (▲) and (3.6) to those obtained for *trans*-$[PtCl_4(cha)(NH_3)]$ (**2**) (□), *cis*-$[PtCl_4(cha)(NH_3)]$ (**3**) (♦), and *cis*-$[PtCl_4(NH_3)_2]$ (**4**) (○)

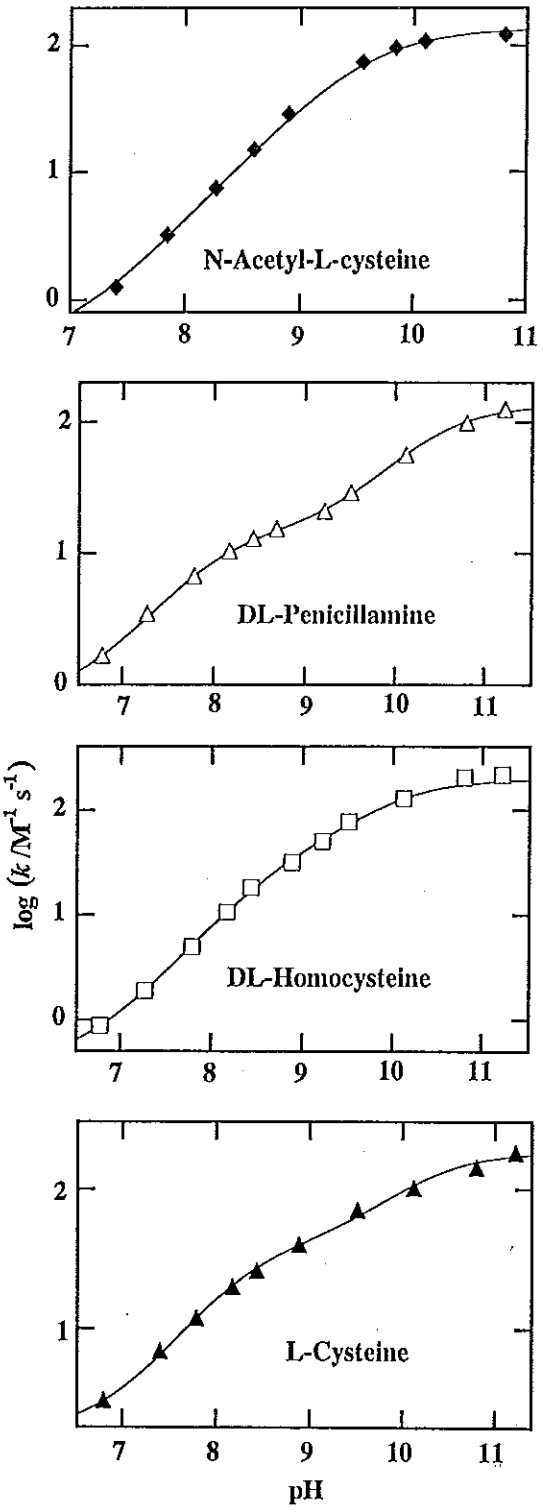


Figure 3.11 Plots of second-order rate constants k as a function of pH for reduction of *trans, trans, trans*-$[PtCl_2(OH)_2(NH_3)(cha)]$ (**5**) by L-cysteine (▲), DL-homocysteine (□), DL-penicillamine (Δ), and N-acetyl-L-cysteine (♦) at 25 °C and I = 1.0 M. The solid lines represent the fits of Eqns. (3.7) (▲, □, Δ) and (3.8) (♦) to experimental data

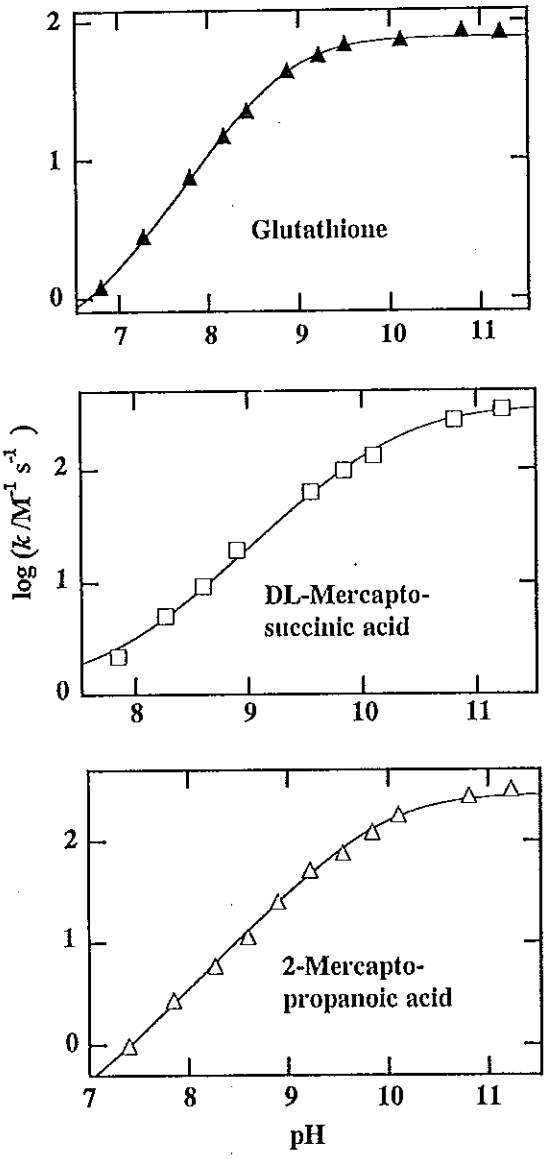


Figure 3.12 Plots of second-order rate constants k as a function of pH for reduction of *trans, trans, trans*-$[PtCl_2(OH)_2(NH_3)(cha)]$ (**5**) by 2-mercaptopropanoic acid (Δ), DL-mercaptosuccinic acid (□), and glutathione (▲) at 25 °C and I = 1.0 M. The solid lines represent the fits of Eqns. (3.8) (Δ), (3.9) (□) and (3.10) (▲) to experimental data

3.4.2 Calculation of Rate Constants for Reduction of Compounds 4, 7 – 9, and 11 - 15 by L-Ascorbic Acid

Reduction of platinum(IV) compounds by ascorbic acid is pH sensitive since the relative concentrations of the reductant species H_2Asc, $HAsc^-$, and Asc^{2-} vary with pH (Fig. 3.13). Eqn. (3.11) is derived from Scheme 3.6 where k_2 and k_3 denote the second-order rate constants for reduction of the platinum(IV) complexes by $HAsc^-$ and Asc^{2-}, respectively, and K_{a1} and K_{a2} the acid dissociation constants of ascorbic acid (Table 1.2). Reduction by the undissociated ascorbic acid H_2Asc was not observed.

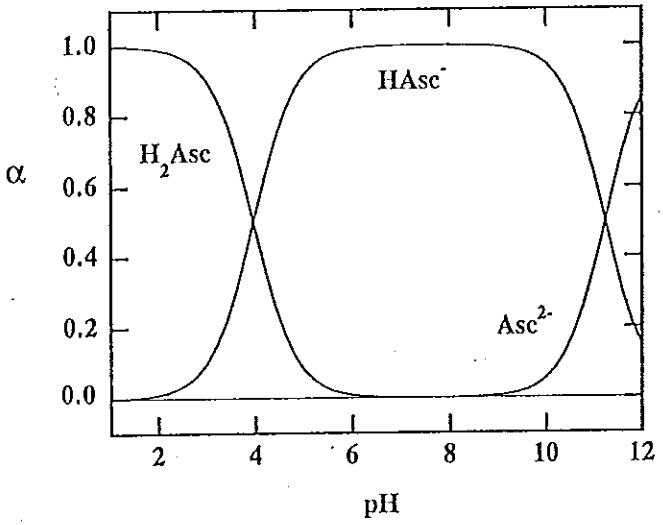


Figure 3.13 Distribution of the protolytic species of ascorbic acid as a function of pH in aqueous solution at 25 °C and I = 1.0 M

$$k = \frac{k_2 K_{a1} a_H + k_3 K_{a1} K_{a2}}{a_H^{\,2} + K_{a1} a_H + K_{a1} K_{a2}} \qquad (3.11)$$

Eqn. (3.11) is fitted to the experimental data obtained for compounds **4**, **9**, and **11** – **14**. Data analysis for these compounds was made in the region $4.0 \leq pH \leq 7.2$. A separate nonlinear least-squares analysis was made for compound **13** in the region $2.25 \leq pH \leq 7.20$. The rate constants k_2 and k_3 obtained from analysis of the two regions are in excellent agreement (Table 3.9) indicating that the former pH interval is wide enough to determine the

The pH-profiles for reduction of the model and anticancer active platinum(IV) compounds by ascorbic acid are displayed Figs. (3.14) - (3.16) and the second-order rate constants k_2 and k_3 derived from the curve fits are summarized in Table 3.9.

Since $HAsc^-$ is the predominant species above pH 5.0, one would expect an almost pH-independent reaction in the region $5.00 \leq pH \leq 7.50$. However, the kinetics data presented in Table 3.7 clearly show that the redox reactions are pH sensitive in this region as

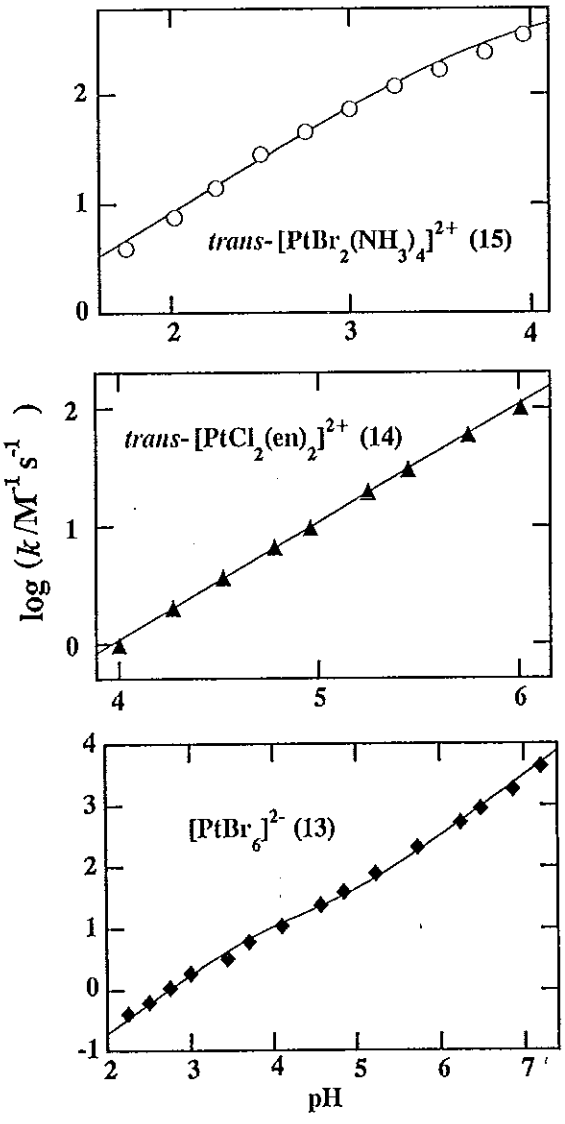


Figure 3.14 Plots of second-order rate constants k as function of pH for reduction of $[PtBr_6]^{2-}$ (♦), *trans*-$[PtBr_2(NH_3)_4]^{2+}$ (○), and *trans*-$[PtCl_2(en)_2]^{2+}$ (▲) by ascorbic acid at 25 °C and I = 1.0 M. The solid lines represent the fits of Eqns. (3.11) (♦, ▲) and (3.13) (○) to experimental data

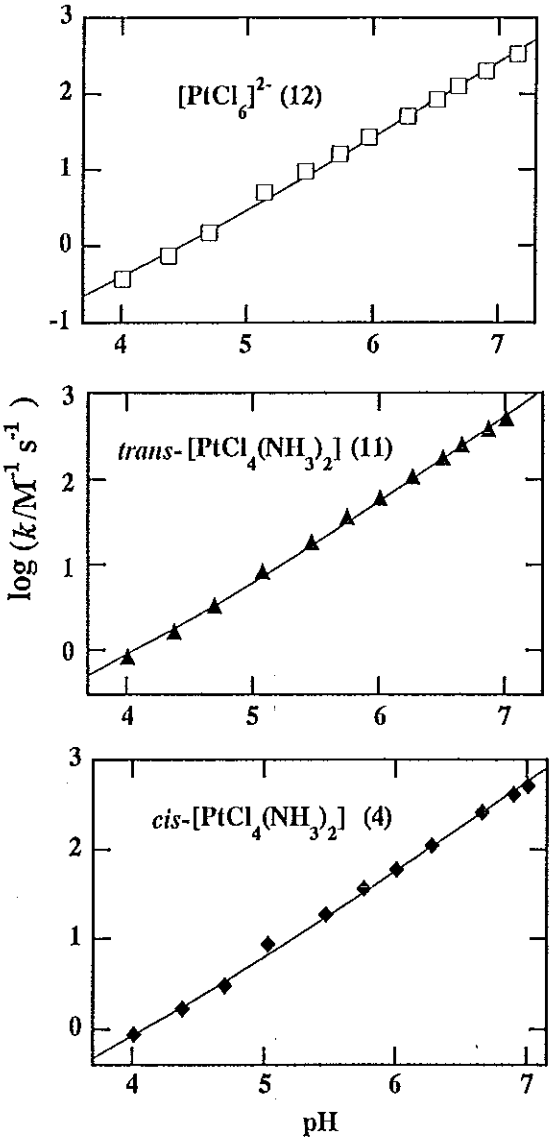


Figure 3.15 Plots of second-order rate constants k as function of pH for reduction of *cis*-$[PtCl_4(NH_3)_2]$ (♦), *trans*-$[PtCl_4(NH_3)_2]$ (▲), and $[PtCl_6]^{2-}$ (□) by ascorbic acid at 25 °C and I = 1.0 M. The solid lines represent the fits of Eqn. (3.11) to the experimental data

cis-[$PtCl_2(OAc)_2$(cha)(NH_3)] (**7**) and *cis, trans, cis*-[$PtCl_2(OCOC_3H_7)_2$(cha)(NH_3)] (**8**) despite the fact that Asc^{2-} constitutes less than 1% of the total concentration of ascorbic acid in the pH region studied, $7.0 \leq pH \leq 7.50$.

Reduction of the model platinum(IV) compounds is slow at lower pH since the Pt(IV)-$HAsc^-$ pathway becomes predominant. The Pt(IV)-Asc^{2-} pathway contributes 97% to the overall rate of reduction at pH 5.74 where the temperature dependence of the second-order overall rate constants k was studied. Therefore, the parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ presented in Table 3.10 are associated with the reduction of the platinum(IV) compounds by ascorbate, Asc^{2-}.

Table 3.5 pH dependence of the second-order overall rate constants k for reduction of compounds **1** - **4** by glutathione at 25 °C and I = 1.0 M

Pt(IV) compound (Reduction product)	pH	$*k$ / M^{-1} s^{-1}
trans-[$PtCl_4(NH_3)$Tz] (**1**)	2.04	3.19 ± 0.06
(*trans*-[$PtCl_2(NH_3)$Tz])	2.32	5.55 ± 0.03
	2.50	7.6 ± 0.3
	2.77	12.80 ± 0.06
	3.00	19.3 ± 0.1
	3.24	34.9 ± 0.2
	3.51	50.9 ± 0.3
	3.75	91.0 ± 0.4
	3.98	159 ± 2
	4.25	284 ± 1
	4.50	530 ± 14
	4.75	901 ± 11
	4.98	(1.50 ± 0.03) x 10^3
trans-[$PtCl_4(NH_3)$(cha)] (**2**)	4.50	73 ± 1
(*trans*-[$PtCl_2(NH_3)$(cha)])	4.67	113 ± 2

to be continued

Table 3.5 continued

	5.00	220 ± 10
	5.37	560 ± 10
	5.68	(1.25 ± 0.03) x 10^3
	5.87	(2.00 ± 0.04) x 10^3
	6.23	(4.29 ± 0.08) x 10^3
	6.36	(6.35 ± 0.14) x 10^3
	6.61	(1.02 ± 0.03) x 10^4
	6.80	(1.46 ± 0.08) x 10^4
	6.90	(1.84 ± 0.09) x 10^4
cis-[$PtCl_4(NH_3)$(cha)] (**3**)	4.46	30 ± 1
(*cis*-[$PtCl_2(NH_3)$(cha)])	4.70	55 ± 2
	5.00	115 ± 3
	5.37	251 ± 6
	5.70	605 ± 7
	5.90	960 ± 20
	6.22	(2.07 ± 0.01) x 10^3
	6.45	(3.64 ± 0.05) x 10^3
	6.72	(6.31 ± 0.17) x 10^3
	6.88	(9.07 ± 0.28) x 10^3
cis-[$PtCl_4(NH_3)_2$] (**4**)	4.55	27 ± 1
(*cis*-[$PtCl_2(NH_3)_2$])	4.85	50 ± 2
	5.05	86 ± 2
	5.43	223 ± 7
	5.90	620 ± 20
	6.23	(1.37 ± 0.02) x 10^3

to be continued

Table 3.5 continued

	6.46	$(2.33 \pm 0.04) \times 10^3$
	6.64	$(3.44 \pm 0.10) \times 10^3$
	6.88	$(5.87 \pm 0.07) \times 10^3$

* Errors are given as one standard deviation

Table 3.6 pH dependence of the second-order overall rate constants *k* for reduction of *t, t, t*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**) by thiols at 25 °C and $I = 1.0$ M

Thiol	pH	$^{\dagger}k/M^{-1}\ s^{-1}$
L-cysteine	6.80	3.10 ± 0.03
	7.40	7.0 ± 0.2
	7.79	11.9 ± 0.3
	8.17	19.8 ± 0.1
	8.43	25.7 ± 0.3
	8.88	39.7 ± 0.5
	9.51	70.4 ± 0.4
	10.12	102 ± 1
	10.80	143 ± 1
	11.22	182 ± 1
DL-penicillamine	6.78	1.66 ± 0.01
	7.27	3.5 ± 0.1
	7.79	6.8 ± 0.1
	8.17	10.4 ± 0.1
	8.43	12.9 ± 0.1

to be continued

Table 3.6 continued

	8.69	15.3 ± 0.3
	9.22	20.9 ± 0.1
	9.51	29.0 ± 0.7
	10.12	56.3 ± 2.4
	10.80	99 ± 1
	11.22	126 ± 1
DL-Homocysteine	6.78	0.88 ± 0.05
	7.27	1.89 ± 0.04
	7.79	4.92 ± 0.05
	8.17	10.7 ± 0.1
	8.43	18.4 ± 0.1
	8.88	31.8 ± 0.3
	9.22	50.5 ± 0.2
	9.51	77.6 ± 0.7
	10.12	129.7 ± 0.7
	10.80	205 ± 6
	11.22	217 ± 4
N-Acetyl-L-cysteine	7.40	1.26 ± 0.02
	7.85	3.20 ± 0.02
	8.27	7.4 ± 0.2
	8.60	15.0 ± 0.2
	8.90	28.5 ± 0.8
	9.55	73.8 ± 1.4
	9.84	96 ± 2
	10.10	109 ± 1

to be continued

Table 3.6 continued

	10.81	123 ± 1
2-Mercaptopropanoic acid	7.85	2.72 ± 0.07
	8.27	5.9 ± 0.2
	8.60	11.5 ± 0.1
	8.90	25.4 ± 0.1
	9.22	51.8 ± 0.2
	9.55	76.5 ± 0.4
	9.84	122 ± 2
	10.10	180 ± 2
	10.81	276 ± 6
	11.22	322 ± 4
DL-Mercaptosuccinic acid	7.85	2.20 ± 0.02
	8.27	5.05 ± 0.01
	8.60	9.3 ± 0.1
	8.90	19.3 ± 0.3
	9.55	63 ± 1
	9.84	97.7 ± 0.4
	10.10	133 ± 1
	10.81	272 ± 3
	11.22	339 ± 2
Glutathione	6.80	1.21 ± 0.01
	7.27	2.81 ± 0.06
	7.33	5.0 ± 0.2[¶]
	7.79	7.54 ± 0.07
	8.17	14.87 ± 0.05

to be continued

Table 3.6 continued

	8.43	22.4 ± 0.3
	8.88	43.6 ± 0.2
	9.22	56.8 ± 0.1
	9.51	68.1 ± 0.5
	10.12	73.5 ± 0.4
	10.80	85 ± 2
	11.22	82 ± 3

[†] Errors are given as one standard deviation.
[¶] Measured at 37 °C, [Pt(IV)] = 0.14 mM, [GSH] = 6.0 mM

Table 3.7 pH dependence of the second-order overall rate constants k for reductions of platinum(IV) compounds by ascorbic acid at 25 °C and I = 1.0 M

Pt(IV) compound (Reduction product)	pH	[§] k/M^{-1} s^{-1}
cis-$[PtCl_4(NH_3)_2]$ (**4**)	4.01	0.875 ± 0.022
(*cis*-$[PtCl_2(NH_3)_2]$)	4.38	1.68 ± 0.03
	4.70	3.00 ± 0.03
	5.03	8.6 ± 0.1
	5.47	18.6 ± 0.2
	5.76	36.9 ± 0.3
	6.01	59.8 ± 0.2
	6.28	111.3 ± 0.2
	6.66	262 ± 3
	6.90	412 ± 2

to be continued

Table 3.7 continued

	7.01	512 ± 2
	7.16	675 ± 9
c, t, c-[$PtCl_2(OAc)_2(cha)(NH_3)$] (**7**)*	7.00	0.083 ± 0.005
(*cis*-([$PtCl_2(cha)(NH_3)$])	7.12	0.100 ± 0.003
	7.25	0.142 ± 0.005
	7.38	0.18 ± 0.01
	7.52	0.25 ± 0.01
c, t, c-[$PtCl_2(OCOC_3H_7)_2(cha)(NH_3)$] (**8**)*	7.00	0.047 ± 0.003
(*cis*-[$PtCl_2(cha)(NH_3)$])	7.12	0.059 ± 0.001
	7.25	0.083 ± 0.001
	7.38	0.109 ± 0.003
	7.52	0.17 ± 0.01
c,t,c-[$Pt(OAc)_2Cl_2(cha)(NH_3)$] (**9**)	4.00	0.420 ± 0.004
(*cis*-[$Pt(OAc)_2(cha)(NH_3)$])	4.25	0.657 ± 0.003
	4.50	1.13 ± 0.01
	4.77	1.69 ± 0.02
	5.00	3.00 ± 0.01
	5.26	4.79 ± 0.02
	5.50	8.27 ± 0.04
	5.98	28.1 ± 0.4
	6.28	48.5 ± 0.6
	6.50	90 ± 1
	6.66	118 ± 2
	6.86	186 ± 4
	7.00	230 ± 6

to be continued

Table 3.7 continued

t,t,t-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**10**)	4.50	0.532 ± 0.011
	5.26	1.92 ± 0.02
	6.28	22.0 ± 0.2
trans-$[PtCl_4(NH_3)_2]$ (**11**)	4.01	0.827 ± 0.022
(*trans*-$[PtCl_2(NH_3)_2]$)	4.38	1.64 ± 0.03
	4.70	3.28 ± 0.04
	5.08	8.2 ± 0.1
	5.47	18.4 ± 0.2
	5.75	36.5 ± 0.5
	6.01	60.9 ± 0.3
	6.27	108 ± 1
	6.51	180 ± 1
	6.66	258 ± 2
	6.87	389 ± 3
	7.01	518 ± 2
	7.16	681 ± 8
$[PtCl_6]^{2-}$ (**12**)	4.01	0.37 ± 0.01
($[PtCl_4]^{2-}$)	4.38	0.74 ± 0.01
	4.70	1.48 ± 0.02
	5.14	5.01 ± 0.04
	5.47	9.5 ± 0.2
	5.74	16.1 ± 0.1
	5.97	26.9 ± 0.1
	6.28	50.9 ± 0.4
	6.51	84.6 ± 0.6

to be continued

Table 3.7 continued

	6.68	127.1 ± 0.4
	6.90	201 ± 1
	7.15	339 ± 2
$[PtBr_6]^{2-}$ **(13)**	2.25	0.404 ± 0.004
($[PtBr_4]^{2-}$)	2.50	0.63 ± 0.01
	2.75	1.10 ± 0.02
	3.00	1.85 ± 0.02
	3.44	3.25 ± 0.08
	3.70	6.04 ± 0.05
	4.10	11.1 ± 0.1
	4.35	14.3 ± 0.2
	4.57	23.9 ± 0.2
	4.85	38.8 ± 0.3
	5.06	56.0 ± 0.2
	5.23	77.6 ± 0.7
	5.74	209 ± 1
	6.25	532 ± 4
	6.49	902 ± 11
	6.87	(1.82 ± 0.02) x 10^3
	7.20	(4.32 ± 0.13) x 10^3
trans-$[PtCl_2(en)_2]Cl_2$ **(14)**	4.00	0.968 ± 0.011
($[Pt(en)_2]^{2+}$)	4.27	2.00 ± 0.04
	4.52	3.61 ± 0.01
	4.78	6.58 ± 0.02
	4.96	9.7 ± 0.2

to be continued

Table 3.7 continued

	5.25	19.3 ± 0.6
	5.45	29.74 ± 0.64
	5.75	58.6 ± 0.6
	6.01	100 ± 1
trans-$[PtBr_2(NH_3)_4]Br_2$ **(15)**	1.75	3.93 ± 0.07
$([Pt(NH_3)_4]^{2+})$	2.02	7.6 ± 0.1
	2.25	14.0 ± 0.2
	2.50	28.32 ± 0.06
	2.75	45.5 ± 0.6
	3.00	73.3 ± 0.8
	3.25	118.5 ± 3.1
	3.50	168.0 ± 1.7
	3.75	242 ± 3
	3.96	349 ± 1

[§] Errors are given as one standard deviation; * Measured at 35 °C

Table 3.8 Second-order rate constants for reduction of the anticancer active Pt(IV) compounds by the various protolytic species of the thiols at 25 °C and I = 1.0 M

Pt(IV) compound	Thiol	$k_i/M^{-1}\ s^{-1}$
trans-[$PtCl_4(NH_3)Tz$] (**1**)	Glutathione	$k_2 = 6.23$
		$k_3 = 56.3 \pm 0.4$
		$k_4 = (1.43 \pm 0.01) \times 10^7$
trans-[$PtCl_4$(cha)(NH_3)] (**2**)	Glutathione	$k_4 = (3.86 \pm 0.03) \times 10^6$
cis-[$PtCl_4$(cha)(NH_3)] (**3**)	Glutathione	$k_4 = (1.83 \pm 0.01) \times 10^6$
cis-[$PtCl_4(NH_3)_2$] (**4**)	Glutathione	$k_4 = (1.18 \pm 0.01) \times 10^6$
†*trans*-[$PtCl_2(CN)_4]^{2-}$	Glutathione	$k_2 = 23.4 \pm 0.3$
		$k_3 = 655 \pm 4$
		$k_4 = (1.10 \pm 0.01) \times 10^8$
t,t,t-[$PtCl_2(OH)_2$(cha)(NH_3)] (**5**)	Glutathione	$k_3 = 0.48 \pm 0.01$
		$k_4 = 111 \pm 1$
		$k_5 = 76.0 \pm 0.4$
	L-Cysteine	$k_2 = 1.77 \pm 0.03$
		$k_3 = 56.4 \pm 0.3$
		$k_4 = 184 \pm 1$
	DL-Homocysteine	$k_2 = 0.37 \pm 0.03$
		$k_3 = 88.5 \pm 0.4$
		$k_4 = 197 \pm 1$
	DL-Penicillamine	$k_2 = 0.74 \pm 0.01$
		$k_3 = 19.3 \pm 0.1$
		$k_4 = 136 \pm 1$
	N-Acetyl-L-cysteine	$k_2 = 0.40 \pm 0.02$
		$k_3 = 138.6 \pm 0.7$
	2-Mercaptopropionic acid	$k_2 = 0.122 \pm 0.006$
		$k_3 = 290 \pm 1$
	DL-Mercaptosuccinic acid	$k_3 = 1.283 \pm 0.013$
		$k_4 = 362 \pm 1$

† ref 157

Table 3.9 Second-order rate constants for reduction of Pt(IV) compounds by hydrogen ascorbate (k_2) and ascorbate (k_3) at 25 (**4**, **9**, **11- 15**) and 35 °C (7, **8**) and I = 1.0 M

Pt(IV) compound	k_2/M^{-1} s^{-1}	k_3/M^{-1} s^{-1}	$^{\dagger}E_{cath}$/mV
cis-$[PtCl_4(NH_3)_2]$ (**4**)	1.07 ± 0.02	(9.83 ± 0.01) x 10^6	-150 (10)
c, t, c-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (7)	-	886 ± 14 (672)*	-300 (30)
c, t, c-$[PtCl_2(OCOC_3H_7)_2(cha)(NH_3)]$ (**8**)	-	524 ± 4 (428)*	-200 (30)
t ,c, c-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**9**)	0.548 ± 0.004	(4.46 ± 0.01) x 10^6	-40 (20)
t, t, t-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**10**)	0.3 *	2 x 10^{6*}	-10 (20)
trans-$[PtCl_4(NH_3)_2]$ (**11**)	1.15 ± 0.02	(9.45 ± 0.02) x 10^6	-190 (10)
$K_2[PtCl_6]$ (**12**)	0.50 ± 0.01	(4.63 ± 0.08) x 10^6	
$K_2[PtBr_6]$ (**13**)	17.51 ± 0.07	(5.35 ± 0.02) x 10^7	
	17.82 ± 0.14‡	(5.35 ± 0.02) x $10^{7\ddagger}$	
trans-$[PtCl_2(en)_2]Cl_2$ (**14**)	1.00 ± 0.02	(1.88 ± 0.08) x 10^7	-30 (10)
trans-$[PtBr_2(NH_3)_4]Br_2$ (**15**)	764 ± 1	-	+70 (10)

* Estimated values at 25 °C ; † ref [190]: Conditions: r.t., pH 7.0, 0.1 M $CF_3SO_3^-$, SCE, glassy carbon, and platinum-wire electrodes, ‡from analysis of data in the region 4.0 ≤ pH ≤ 7.20

Table 3.10 Second-order rate constants k and activation parameters for reduction of model and anticancer active platinum(IV) compounds by ascorbate Asc^{2-} at pH 5.74 (**4**, **11** - **14**), 6.28 (**9**, **10**), and 7.12 (**7**, **8**) and $I = 1.0$ M

Pt(IV) compound	T/°C	k/M^{-1} s^{-1}	$\Delta H^{\ddagger}$ /kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}
cis-$[PtCl_4(NH_3)_2]$ (**4**)	20	23.0 ± 0.2	60 ± 2	-14 ± 7
	25	36.9 ± 0.3		
	30	56.1 ± 0.2		
	35	79.8 ± 0.8		
c,t,c-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**7**)	20	$(3.34 \pm 0.09) \times 10^{-2}$	52 ± 1	-97 ± 4
	25	$(5.08 \pm 0.02) \times 10^{-2}$		
	30	$(7.23 \pm 0.01) \times 10^{-2}$		
	35	0.100 ± 0.003		
	40	0.140 ± 0.007		
c,t,c-$[PtCl_2(OCOC_3H_7)_2(cha)(NH_3)]$ (**8**)	25	$(3.25 \pm 0.05) \times 10^{-2}$	46 ± 1	-120 ± 4
	30	$(4.4 \pm 0.1) \times 10^{-2}$		
	35	$(5.94 \pm 0.05) \times 10^{-2}$		

to be continued

3.5 Reaction Mechanism

3.5.1 Mechanism for Reduction of the Anticancer Active Platinum(IV) Compounds 1 – 5 by Thiols

Reduction of the anticancer active compounds **1** - **4** by glutathione and of **5** by the thiols is proposed to follow a halide-bridged mechanism involving a reductive attack by the reducing agent on a halide coordinated *trans* to a halide. Halide-bridged reductive elimination reactions of platinum(IV) compounds can be visualized as processes involving the movement of the bridging ligand from the oxidant towards reductant and a simultaneous movement of the *trans* ligand (leaving group) away from the oxidant in the opposite direction during the course of reaction [160]. Since the two electrons transferred to the platinum(IV) compound would be accepted in the $d_{z}2$ orbital in the transition state, the availability of this orbital would be increased by an outward movement of the *trans* ligand. It is then expected that for strong-field *trans* ligands such as NH_3 and OH^-, the activation process is energetically unfavorable.

Since platinum(IV) compounds are generally substitution inert, and the pseudo-first-order rate constants k_{obsd} are not influenced by free chloride, substitution-controlled inner-sphere electron transfer is unlikely. Previous studies on reductions of platinum(IV) halide complexes by inorganic [144,155,156,159,160,191-193] and biological [157,158] reductants have shown that electron transfer takes place through a reductive attack by the reductant on a halide coordinated *trans* to good leaving groups forming a halide-bridged activated complex. Reductive elimination reactions of platinum(IV) compounds taking place by this mechanism are formally equivalent to transfer of X^+ (X = Cl, Br) from the Pt(IV) center to the reducing molecule/ion [144,155-160,191-193]. The detection of a BrCN intermediate for reduction of *trans*-$[PtBr(CN)_4(OH)]^{2-}$ by CN^- gives support for the transfer of Br^+ to CN^- [160].

Electron transfer from glutathione to the platinum(IV) compounds **1- 4** takes place along the axis Cl-Pt-Cl where one of the two chloride ligands is eliminated as Cl^- and the other is transferred to GS^- as Cl^+ leading to the formation of the platinum(II) product and the intermediate GSCl. The chloride ligands coordinated *trans* to ammonia, cyclohexylamine,

and thiazole cannot play the role of bridging electron transfer since the amine ligands are not good leaving groups.

An important question regarding the intimate mechanism for reduction of *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**) is whether reaction takes place *via* an attack on chloride or hydroxide ligands. Although both hydroxide and chloride are potential bridging ligands, electron transfer is expected to take place through the axis Cl-Pt-Cl, *i.e.* by an attack of GS^- and/or GSH on one of the chloride ligands. This is reasonable in view of the fact that heavier and more polarizable ligands are effective in bridging [160]. Transfer of OH^+, in the case of hydroxide-bridged electron transfer, from Pt(IV) to the reducing thiol or thiolate is unlikely. The product for reduction of **5** by the thiols is identified to be *trans*-$[Pt(OH)_2(cha)(NH_3)]$ (*cf.* Fig. 3.2) confirming that electron transfer takes place by a chloride-bridged pathway which involves an activated complex of the type shown in Fig. 3. 17. A similar transition state is envisaged for reduction of compounds **1** – **4** by glutathione.

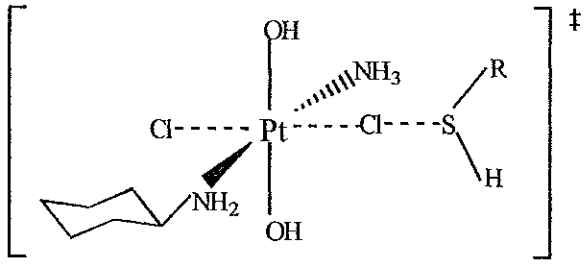


Figure 3.17 A chloride-bridged transition state formulated for the reduction of *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**) by the thiols

Noteworthy, the *cis* congener of compound **5**, *viz. cis, trans, cis*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**6**) which has been reported to have a lower cytotoxicity than **5** [68], does not undergo reduction under the conditions used for reduction of compound **5**. This is not unexpected since reduction of compound **6** by a chloride-bridged pathway is not energetically favourable for the reason that both ammonia and cyclohexylamine are not good

leaving groups. However, thiol reduction of **6** may occur at much longer time scale than that observed for compound **5**, and probably by a different mechanism.

The intermediate oxidation product RSCl undergoes the rapid subsequent reactions (3.14) – (3.16) to produce RSSR as the final oxidation product [194]. From the observation that there is a large difference between the redox reactivities of RSH and RS^- it is inferred that the thiols attack coordinated chloride through the sulfur atom. Deprotonation of the

$$RSCl + H_2O \rightarrow RSOH + Cl^- + H^+ \quad (3.14)$$

$$RSOH + RSH \rightarrow RSSR + H_2O \quad (3.15)$$

$$RSOH + RS^- \rightarrow RSSR + OH^- \quad (3.16)$$

carboxylic groups does not increase the reactivity of thiols significantly while that of the SH group results in a substantial increase in reactivity. For instance, it can be seen from the data presented in Table 3.8 that GS^- is five orders of magnitude more reactive than its protonated form GSH towards oxidation by *trans*-$[PtCl_4(NH_3)Tz]$ (**1**) and *trans*-$[PtCl_2(CN)_4]^{2-}$. It follows from this that the reactivity of the thiols is largely governed by their proton basicity. In support of this, a roughly linear correlation between the rate constants k_{RS^-} for reduction of **5** by thiolate species RS^- and the acid dissociation constants K_{RSH} for the corresponding thiol RSH, defined by Eqn. (3.17). The plot of $\log k_{RS^-}$ *vs.* pK_{RSH} is shown in Fig. (3.18).

$$\log k_{RS^-} = (0.52 \pm 0.06)\, pK_{RSH} - (2.8 \pm 0.5) \quad (3.17)$$

It can be seen from Fig. 3.18 that the reactivity of the thiolate species RS^- is directly related to their proton basicity. Glutathione, which is a bulky thiol and penicillamine, which has the largest cone angle [195] , do not show any significant deviation from the correlation indicating that steric effects on the reactivity of the thiols play a minor role. This is in line with the proposed structure of the activated complex (*cf.* Fig. 3.17) which is formed by a reductive attack of the thiols on a coordinated chloride rather than directly on the Pt(IV) center, in which case steric factors are expected to be significant. The slightly negative

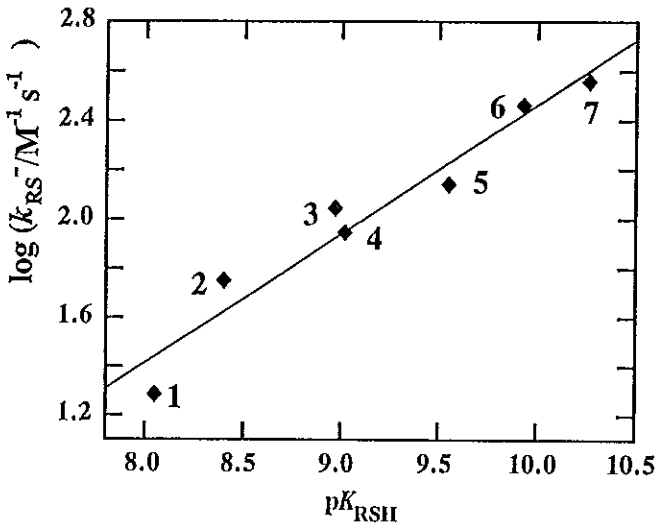


Figure 3.18 Correlation of the rate constants k_{RS^-} for reduction of *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ by thiolate species RS^- with the acid dissociation constants K_{RSH} of the thiols. The numbers **1** – **7** represent penicillamine, cysteine, glutathione, homocysteine, N-acetyl-L-cysteine, 2-mercaptopropanoic acid, and 2-mercaptosuccinic acid, respectively

deviation of penicillamine from the correlation indicates that steric crowding near the reaction site, rather than the overall bulkiness of thiol, affects reactivity.

There is a striking mechanistic similarity between reduction of platinum(IV) halide compounds by thiols and thiol-disulfide exchange reactions [196], which occur *via* a simple nucleophilic displacement (S_N2) of a thiolate anion $R'S^-$ from disulfide $R'SSR'$ by another thiolate anion RS^- according to equation (3.18) [196-200]. A protonated thiol RSH is not reactive towards $R'SSR'$ which parallels the present observation that reduction of the

$$R'SSR' + RS^- = RSSR' + R'S^-, (k_+, k_-) \qquad (3.18)$$

anticancer active compounds **1** – **4** by GSH was not observed. Linear Brønsted correlations between the rate constant k_+ and the acid dissociation constant K_{RSH} defined by $\log k_+ = \alpha\, pK_{RSH} + \beta$ have been reported for thiolate-disulfide exchange reactions [196-200]. Interestingly, the values for α are comparable with the slope of the correlation observed for reduction of **5** by the thiolate species RS^- suggesting the mechanistic similarity between the

3.5.2 Mechanism of Reduction of Platinum(IV) Compounds by Ascorbic Acid

Although ascorbic acid is a well characterised two-electron reductant different mechanisms have been proposed for its reactions with metal complexes [113,114,119,201-206]. Reactions have been classified as (*1*) outer-sphere, (*2*) inner-sphere, as well as (*3*) bridged electron transfer where ascorbate is bound to a co-ordinated ligand prior to electron transfer [119]. When the redox process involves a decrease in coordination number, as in the case of reduction of platinum(IV) to platinum(II), the reactions are can be classified as (*4*) reductive *trans* eliminations.

To date, only few mechanistic studies on the reduction of platinum(IV) compounds by ascorbic acid have been made [207-210]. Bose and Weaver [209] have reported that ascorbic acid reduction of the second-generation anticancer active compound *cis, trans, cis*-$[PtCl_2(OH)_2(i\text{-}PrNH_2)_2]$ (iproplatin) is catalyzed by platinum(II) which is remarkably different from Evans and Green's report that the complex is reduced reversibly by a simple two-electron transfer process involving no catalysis by platinum(II) [208].

The platinum(II)-catalyzed reduction pathway involves the reaction sequence Eqns. (3.19) – (3. 21) whereby ascorbate is substituted for hydroxide and then internal electron transfer according to equations (3.22) – (3.24) [209]; the chloride and amine ligands are omitted for simplicity.

$$Pt(II) + asc = Pt^{II}\text{-}asc, \qquad K_1 \tag{3.19}$$

$$HO\text{-}Pt^{IV}\text{-}OH + Pt^{II}\text{-}asc = HO\text{-}Pt^{IV}\text{-}O\text{-}Pt^{II}\text{-}asc, \qquad K_2 \tag{3.20}$$

$$HO\text{-}Pt^{IV}\text{-}O\text{-}Pt^{II}\text{-}asc \rightarrow Pt(II) + HO\text{-}Pt^{IV}\text{-}asc, \qquad k \tag{3.21}$$

$$HO\text{-}Pt^{IV}\text{-}asc \rightarrow HO\text{-}Pt^{III}\text{-}OH_2 + asc^{\cdot}, \qquad k_3 \tag{3.22}$$

$$Pt^{III} + asc \rightarrow Pt^{II} + asc^{\cdot}, \text{ fast} \tag{3.23}$$

$$2\ asc^{\cdot} \rightarrow asc + dasc, \text{ fast} \tag{3.24}$$

This mechanism is not operative in the present systems since simple pseudo-first-order kinetic traces with no induction period were observed. More importantly, addition of $[PtCl_4]^{2-}$ up to 10-fold excess to a reaction mixture of $[PtCl_6]^{2-}$ (**12**) and ascorbic acid does not affect

the pseudo-first-order rate constant k_{obsd} (k_{obsd} = 0.32 ± 0.01 and 0.325 ± 0.005 s^{-1} for ascorbate reduction of $[PtCl_6]^{2-}$ at pH 6.28 and 25 °C in the absence and presence of 10-fold excess $[PtCl_4]^{2-}$, respectively).

Since platinum(IV) compounds are generally substitution inert, electron transfer preceded by substitution of ascorbate for a coordinated ligand is unlikely. This is confirmed by the facts that the redox reactions are fast, pseudo-first-order in the platinum(IV) complexes, and independent of added chloride or bromide.

Most of the kinetic studies on oxidation of ascorbic acid involved by and large strong outer-sphere one-electron acceptor and substitution inert transition metal complexes with no vacant coordination sites. Since platinum(IV) complexes are coordinatively saturated and substitution inert, electron transfer *via* an outer-sphere mechanism is to be expected. Based on the results of the kinetic studies and the nature of the reduction products electron transfer from ascorbate Asc^{2-} to the platinum(IV) dicarboxylate compounds *cis, trans, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**7**) and *cis, trans, cis*-$[PtCl_2(OCOC_3H_7)_2(cha)(NH_3)]$ (**8**) is proposed to take place by an outer-sphere mechanism. The reduction of $[Pt(NH_3)_6]^{4+}$, which is an outer-sphere oxidant, by ascorbic acid to $[Pt(NH_3)_4]^{2+}$ implies that reduction of platinum(IV) compounds with no potential bridging ligands could follow an outer-sphere mechanism.

Reduction of the model platinum(IV) halide complexes where at least two halide ligands are coordinated *trans* to each other is suggested to occur *via* a halide-bridged reductive *trans* elimination mechanism. This mechanism is similar to that proposed for thiol reduction of compounds **1** – **4** and **5** in that $HAsc^-$ and/or Asc^{2-} undergo a reductive attack on any one of the mutually *trans* coordinated chloride or bromide ligands forming a halide-bridged transition state of the type formulated for compound **10** (Fig. 3.19). In compounds **7** and **8** the chloride ligands are *trans* to ammonia and cyclohexylamine ligands which are not good leaving groups and therefore electron transfer *via* the chloride-bridged mechanism is unfavorable. Electron transfer through acetate- or butryate-bridging is not feasible either

since transfer of OAc^+ or $CH_3CH_2CH_2CO_2^+$ from platinum(IV) to the ascorbate anion is not reasonable.

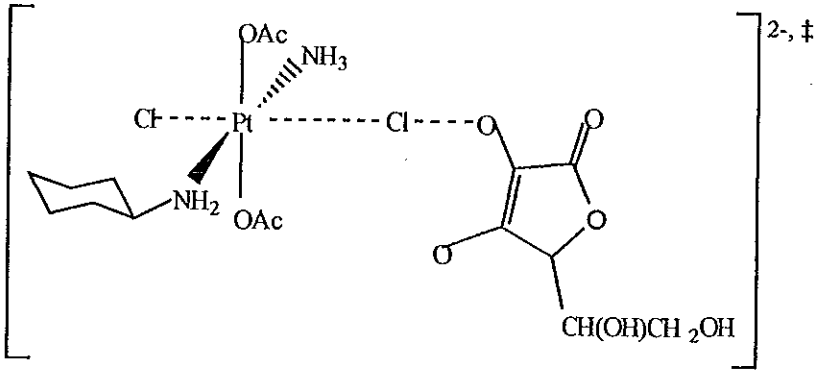


Figure 3.19 Structure of the transition-state complex formulated for ascorbate Asc^{2-} reduction of compound **10**. Ascorbate reductions of the platinum(IV) complexes **4**, **9**, and **11** – **15** are thought to involve transition states of this type

No correlation between the second-order rate constants k_3 and the cathodic reduction potentials E_{cath} for the complexes is observed (Fig. 3.20) suggesting that outer-sphere mechanism is not operative. The compound *trans*-$[PtBr_2(NH_3)_4]^{2+}$ (**15**), which is about three

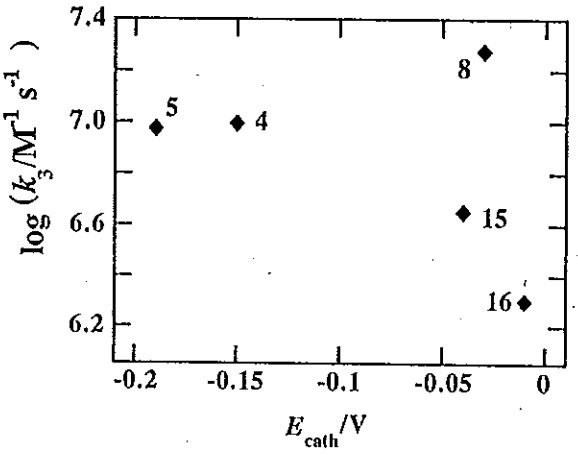


Figure 3.20 Second-order rate constants k_3 for ascorbate Asc^{2-} reductions of compounds **4**, **9** - **11**, and **14** plotted against cathodic reduction potentials E_{cath} from Table 3.9.

orders of magnitude more reactive than its chloride analog *trans*-$[PtCl_2(en)_2]^{2+}$ (**14**), is the most reactive of the model platinum(IV) compounds investigated.

This large difference in reactivity is attributed to the fact that bromide is a more effective bridging ligand than chloride [140,191]. Similarly, reduction of $[PtBr_6]^{2-}$ is ten times faster than that of $[PtCl_6]^{2-}$ despite the fact that the reduction potential of the former is less than that of the latter by about 100 mV.

The product for ascorbate reduction of *trans, trans, trans*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**10**) is observed to be *trans*-$[(OAc)_2(cha)(NH_3)]$ (*cf.* Fig. 3.3) which is expected in the case that electron transfer occurs by a chloride-bridged mechanism. On the other hand, reduction of this compound *via* an outer-sphere mechanism is expected to result in the elimination of the loosely bound acetate ligands instead of chloride. In support of this assumption, a complete displacement of acetate from **10** was observed in the presence of chloride at room temperature (*cf.* Fig. 3.21). The large difference between the rates of reduction of compounds **9** and **7** might suggest that they are reduced by different mechanisms, *viz.* a chloride-bridged mechanism for the former and an outer-sphere mechanism for the latter.

Examination of the kinetic data presented in Table 3.9 reveals that the platinum(IV) compounds with mutually *trans* chloride ligands have similar reactivity but are more reactive than compounds **7** and **8** where the chloride ligands are *trans* to ammonia and cyclohexylamine. The halide ligands which are not directly involved in the electron transfer process have a less remarkable influence on reactivity of the platinum(IV) compounds. This is evident from the fact that *cis*-$[PtCl_4(NH_3)_2]$ (**4**), *trans*-$[PtCl_4(NH_3)_2]$ (**11**), and *trans, cis, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**9**) have comparable rates of reduction regardless of the difference in the spatial distribution and nature of the coordinated ligands. Similarly, it has been reported that the rate of reduction of $[PtBr_2Cl_2(PEt_3)_2]$ by $S_2O_3^{2-}$, in which bromide is the bridging ligand, is only slightly less than that of reduction of $[PtBr_4(PEt_3)_2]$ by the same reductant [146]. It then follows that the four in-plane ligands do not seem to affect the rate of reduction significantly.

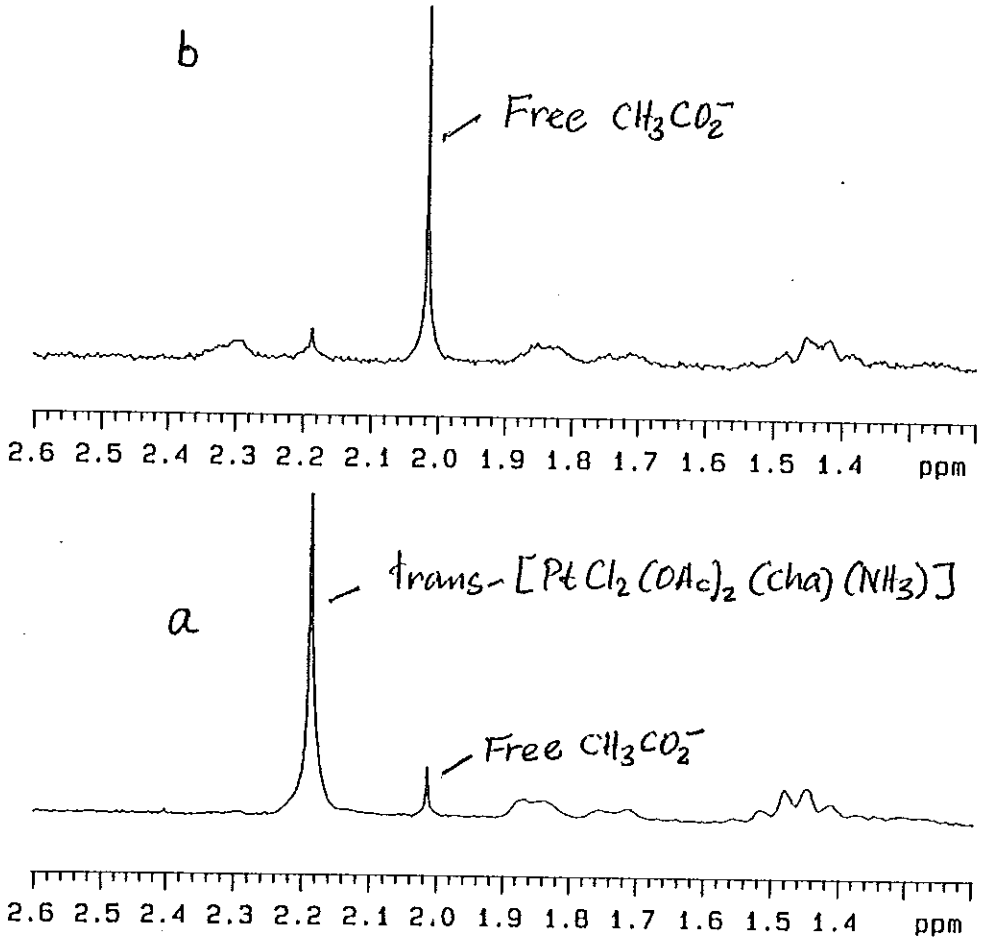


Figure 3.21 Proton NMR spectra of a mixture of 2 mM *trans, trans, trans*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**10**) and 200 mM chloride recorded after 1.5 h (a) and 72 h (b) at room temperature

Further mechanistic information about the mechanism for reduction of the platinum(IV) complexes by ascorbic acid is obtained from the activation parameters presented in Table 3.10. A good *isokinetic relationship* with an isokinetic temperature of 260 K is observed (Fig. 3.22), supporting the conclusion that reduction of the platinum(IV) complexes in which the chloride ligands are coordinated *trans* to each other are reduced by the same mechanism, i.e. a chloride-bridged electron transfer. The deviations of the platinum(IV)

dicarboxylate compounds **7** and **8** suggest that ascorbate reduction of these compounds follows a different mechanism, *i.e.* outer-sphere electron transfer. The small variation of the second-order rate constants (*cf.* Table 3.9) is explained by the observed compensation effect between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$.

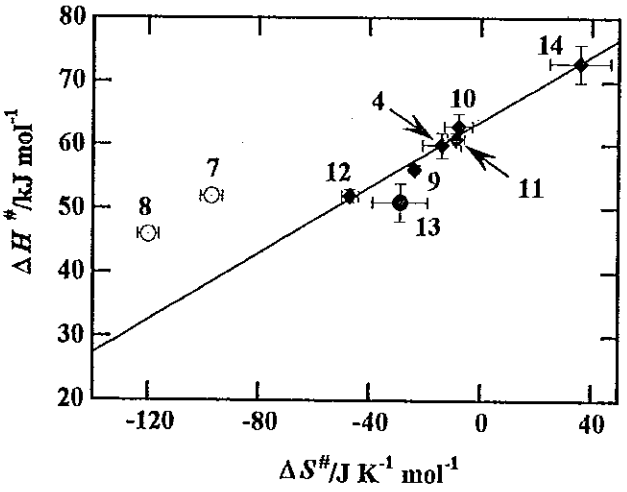


Figure 3. 22 Isokinetic relationship between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for ascorbate Asc^{2-} reduction of the platinum(IV) complexes **4**, and **7** – **14** from Table 3.10.

The redox reactions have negative entropy of activation which is consistent with the fact that electron transfer reactions are normally bimolecular and accompanied by a negative entropy of activation. The positive entropy of activation for *trans*-$[PtCl_2(en)_2]^{2+}$ is not unexpected since there is a neutralization of charge and a considerable de-electrostriction of solvating water on transition state formation. The entropy of activation for *cis, trans, cis*-$[PtCl_2(OAc)_2(NH_3)(cha)]$ **7** is more negative by about 70 J K^{-1} mol^{-1} than that of its isomer *cis, trans, cis*-$[Pt(OAc)_2Cl_2(NH_3)(cha)]$ **9** in accordance with the fact that outer-sphere electron transfer reactions are associated with large decrease in entropy of activation [165]. The unfavourable entropies of activation for compounds **7** and **8** is interpreted to be an indication for the solvation of the leaving carboxylate groups in the activated complex.

- Reductions of the anticancer active tetrachloroplatinum(IV) compounds by glutathione and JM335 by the thiols are pH dependent which is attributed to the variation in the distribution of the protolytic species of the thiols with pH. There is a significant increase in the rate of reduction with increasing pH since the concentrations of the deprotonated thiol species RS^- increase. The thiolate species RS^- is about six orders of magnitude more reactive than RSH suggesting that proton basicity is an important factor governing reactivity. Electron transfer from the thiols to the platinum(IV) complexes takes place by a halide-bridged mechanism involving an attack by the thiols on a chloride coordinated *trans* to another chloride leading to the formation of a bridged transition-state. This mechanism is formally equivalent to transfer of Cl^+ from platinum(IV) to the reducing thiol species.

- Ascorbic acid reduction of the orally active platinum(IV) compounds *cis, trans, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (JM216) and *cis, trans, cis*-$[PtCl_2(OCOC_3H_7)_2(cha)(NH_3)]$ (JM221) in a near neutral aqueous perchlorate medium is relatively slow. These compounds are reduced to the same product *cis*-$[PtCl_2(cha)(NH_3)]$ *via* an outer-sphere mechanism. Ascorbic acid reduction of the platinum(IV) complexes possessing mutually *trans* coordinated halides is rapid and take place by a different mechanism, *viz.* a halide-bridged mechanism involving a reductive attack by Asc^{2-} and/or $HAsc^-$ on one of the halides in the axis Cl-Pt^{IV}-Cl or Br-Pt^{IV}-Br leading to the formation of a bridged transition-state.

- The relative ease of reduction of the platinum(IV) compounds by the thiols and ascorbic acid is observed to be related to the nature and spatial distribution of the anionic ligands around the platinum(IV) center. Reduction of the platinum(IV) halide compounds in which there are at least two mutually *trans* halide ligands is rapid. The halide ligands which are not directly involved in the electron transfer processes appear not to influence the reactivity of the platinum(IV) compounds significantly while coordination of hydroxide

ligands leads to a significant decrease in redox reactivity. Glutathione reduction of *trans*-$[PtCl_4(cha)(NH_3)]$, for instance, is faster than that of *cis*-$[PtCl_4(cha)(NH_3)]$ by only a factor of two but more than 4 orders of magnitude so compared to reduction of *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (JM335). Outer-sphere reduction of the platinum(IV) dicarboxylate compounds **7** and **8** is relatively slow. The half-life for reduction of the orally active drug *cis, trans, cis*-$[PtCl_2(OAc)_2(cha)(NH_3)]$ (**7**) by 5 mM total concentration of ascorbic acid (15-fold excess) at 35 °C and pH 7.4 is observed to be 12 min.

- The kinetic results are consistent with the biological properties of the platinum(IV) complexes. The platinum(IV) complexes which undergo rapid reduction display only limited *in vivo* activity and those which are reduced relatively slowly have a better *in vivo* anticancer activity. For instance, there is a wide difference in anticancer activity between *trans*-$[PtCl_4(cha)(NH_3)]$ (**2**) and *trans, trans, trans*-$[PtCl_2(OH)_2(cha)(NH_3)]$ (**5**) with the latter compound showing some *in vivo* anticancer activity. It follows from this that the rates of reduction of platinum(IV) complexes is an important factor affecting their *in vivo* anticancer activity. The rate of reduction by potential bioreductants has to be low enough for uptake and distribution of the parent Pt(IV) drug and at the same time sufficiently high to achieve reduction to platinum(II) and binding to DNA rather than being excreted intact.

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List of Abbreviations

A	Adenine
C	Cytosine
G	Guanine
GSH	Reduced glutathione
GSSG	Oxidized glutathione
JM	Johnson Matthey
RSSR	Oxidized thiol
1,2-d(GPG)	Intrastrand bifunctional adduct on adjacent guanines
1,2-d(APG)	Intrastrand bifunctional adduct on adjacent adenine and guanine bases
cha	Cyclohexylamine
dach	1,2-diaminecyclohexane
i-$PrNH_2$	Isopropylamine
*L*1210	Leukemia cells
*P*388	Leukemia cells
pip	Piperidine
phen	1,10-phenanthroline
nphen	5-nitro-1,10-phenanthroline
cphen	5-chloro-1,10-phenanthroline
mphen	5-methyl-1,10-phenanthroline

dmphen	5,6-dimethyl-1,10-phenanthroline
quin	Quinoline
Tz	Thiazole
IC_{50}	Concentration of compound required to kill 50 % of cells
ILS	Maximum increase in lifespan produced in mice bearing intraperitoneally implanted tumor
LD_{50}	Dose at which 50 % of animals die

Appendices – Pseudo-first-order rate constants for reductions of platinum(IV) complexes **1** – **4** by glutathione and of **4**, **11** – **15** by ascorbate ions as a function of pH and concentration of the reductants

Appendix I. pH dependence of observed pseudo-first-order rate constants for reduction of *trans*-$[PtCl_4(NH_3)Tz]$ (**1**) at 25 °C and I = 1.0 M[a]

pH	$[GSH]_{tot}$/mM	$10^2 k_{obsd}/s^{-1}$
2.77	3	3.78 ± 0.02
	6	7.67 ± 0.01
	10.5	13.4 ± 0.1
	15	19.8 ± 0.1
3.00	2	3.96 ± 0.03
	4	7.88 ± 0.02
	7	13.6 ± 0.1
3.51	1	5.27 ± 0.04
	2	10.2 ± 0.1
	3.5	17.9 ± 0.1
	5	25.6 ± 0.1
3.75	1	9.0 ± 0.1
	2	17.8 ± 0.1
	5	45 ± 1
	10	91 ± 1
3.98	1	15.6 ± 0.1
	2	30.7 ± 0.2
	5	77 ± 2
	10	159 ± 1
4.25	1	28.9 ± 0.2
	2	55.4 ± 0.3
	3.5	95.8 ± 0.4
	5	143 ± 1
4.50	1	53.3 ± 0.3

to be continued

Appendix I continued

	2	103 ± 1
	3.5	179 ± 3
	5	266 ± 2
4.75	1	94.1 ± 0.3
	2	178 ± 1
	3.5	316 ± 1
	5	453 ± 2
4.98	1	163 ± 1
	2	322 ± 6
	5	800 ± 12
	10	1510 ± 40

[a] $[Cl^-]$ = 0.8 – 0.9 M; $[Na_2H_2edta]$ = 2 mM; λ = 280 nm.
Errors are given as one standard deviation.

Appendix II pH dependence of observed pseudo-first-order rate constants for the reduction of *trans*-$[PtCl_4(cha)(NH_3)]$ (**2**) at 25 °C and I = 1.0 M[b]

pH	$[GSH]_{tot}$/mM	$10^2 k_{obsd}/s^{-1}$
4.50	1.0	8.0 ± 0.1
	2.0	16.8 ± 0.1
	3.5	27.5 ± 0.1
	6.0	46.5 ± 0.2
	10.0	74.5 ± 0.2
4.67	1.0	12.7 ± 0.1
	2.0	25.7 ± 0.1
	3.5	42.2 ± 0.2
	6.0	72.9 ± 0.1
	10.0	114.3 ± 0.7
5.00	1.0	25.1 ± 0.9

to be continued

Appendix II continued

	2.0	53 ± 1
	3.5	91.3 ± 1.2
	6.0	146.0 ± 1.3
	10.0	225 ± 8
5.37	0.4	28.6 ± 0.6
	0.8	55.8 ± 1.1
	1.6	104.0 ± 2.4
	3.0	178.0 ± 1.7
	4.0	233.4 ± 1.3
5.68	0.4	57.5 ± 1.3
	0.8	114.4 ± 0.4
	1.6	221.7 ± 3.3
	2.6	350 ± 2
	4.0	507.0 ± 2.4
5.87	0.4	95.5 ± 0.3
	0.8	183 ± 1
	1.6	356 ± 2
	3.0	634 ±5
	4.0	812 ± 7
6.23	0.3	144 ± 1
	0.6	282 ± 1
	1.2	537 ± 2
	2.0	904 ± 14
	3.0	1295 ± 5
6.36	0.3	184 ± 1
	0.6	390 ± 2
	1.2	786 ± 9
	2.0	1264 ± 11
6.61	0.3	338 ± 3
	0.6	664 ± 6
	1.2	1325 ± 8
	2.0	2075 ± 11

to be continued

Appendix II continued

6.80	0.3	515 ± 3
	0.6	1047 ± 12
	1.2	2016 ± 12
	2.0	3013 ± 20
6.90	0.3	642 ± 2
	0.6	1330 ± 10
	1.2	2508 ± 6
	2.0	3791 ± 25

[b] $[Cl^-]$ = 0.8 M; $[Na_2H_2edta]$ = 0.5 mM; λ = 240 nm. Errors are given as one standard deviation.

Appendix III pH dependence of observed pseudo-first-order rate constants for reduction of *cis*-$[PtCl_4(cha)(NH_3)]$ (**3**) at 25 °C and I = 1.0 M[c]

PH	$[GSH]_{tot}$/ mM	$10^2 k_{obsd}$/ s^{-1}
4.46	1.5	4.8 ± 0.3
	3.0	9.5 ± 0.1
	6.0	18.1 ± 0.1
	10	29.5 ± 0.1
	15	45.9 ± 0.4
4.70	1.5	11 ± 0.2
	3.0	21.3 ± 0.1
	6.0	35.8 ± 0.3
	10	58.9 ± 0.3
5.00	0.5	6.4 ± 0.1
	1.25	16.1 ± 0.2
	2.0	24.7 ± 0.3
	3.5	43.8 ± 0.5
	6.0	69.6 ± 0.5
5.37	0.5	14.9 ± 0.1

to be continued

Appendix III continued

	1.0	29.5 ± 0.1
	2.0	56.4 ± 0.4
	3.0	81.7 ± 0.2
	5.0	128 ± 1
5.70	0.5	41.3 ± 0.3
	1.0	70.9 ± 0.4
	2.0	133.4 ± 0.2
	3.4	216.3 ± 0.3
5.90	0.3	30.9 ± 0.1
	0.6	61.1 ± 0.2
	1.2	125.0 ± 0.2
	2.0	198 ± 1
	3.0	291 ± 1
6.22	0.3	78.3 ± 0.3
	0.6	139.0 ± 0.4
	1.2	260 ± 2
	2.0	430 ± 2
	3.0	635.3± 2.2
6.45	3.0	116.0 ± 0.4
	0.6	233 ± 1
	1.2	464 ± 2
	2.0	753 ± 5
	3.0	1098 ± 8
6.72	0.3	204 ± 19
	0.6	430 ± 4
	1.2	839 ± 7
	2.0	1340 ± 10
	3.0	1911 ± 11
6.88	0.3	272 ± 1
	0.6	581 ± 4
	1.2	1151 ± 8
	2.0	1819 ± 7

[c] $[Cl^-] = 0.7$ M; $[Na_2H_2edta] = 0.5$ mM; $\lambda = 240$ nm. Errors are given as one standard deviation.

Appendix IV continued

	5.0	1175 ± 14
6.64	0.4	144.7 ± 1.2
	0.8	293.6 ± 1.4
	1.6	603.9 ± 2.3
	2.6	951 ± 7
	4.0	1378 ± 2
6.88	0.4	243 ± 1
	0.8	493 ± 3
	1.6	967 ± 18
	2.6	1536 ± 9

[d] $[Cl^-] = 0.2$ M; $[Na_2H_2edta] = 0.5$ mM; $\lambda = 240$ nm. Errors are given as one standard deviation.

Appendix V pH dependence of observed pseudo-first-order rate constants for ascorbic acid reduction of *cis*-$[PtCl_4(NH_3)_2]$ (**4**) at 25 °C and $I = 1.0$ M[¶]

pH	$[H_2Asc]_{tot}$/mM	k_{obsd}/s^{-1}
5.03	2.5	$(2.40 \pm 0.01) \times 10^{-2}$
	5.0	$(4.60 \pm 0.01) \times 10^{-2}$
	10	$(8.80 \pm 0.01) \times 10^{-2}$
	17	0.145 ± 0.001
	25	0.218 ± 0.002
5.47	2	$(4.12 \pm 0.07) \times 10^{-2}$
	4	0.798 ± 0.007
	8	0.152 ± 0.001
	13	0.242 ± 0.001
	20	0.378 ± 0.003
5.76	2	$(8.06 \pm 0.06) \times 10^{-2}$
	4	0.153 ± 0.001
	8	0.295 ± 0.003

to be continued

Appendix V continued

	13	0.488 ± 0.002
	20	0.743 ± 0.007
6.01	1.5	$(9.6 \pm 0.1) \times 10^{-2}$
	3	0.188 ± 0.002
	6	0.362 ± 0.003
	10	0.607 ± 0.003
	15	0.90 ± 0.01
6.28	2	0.234 ± 0.002
	4	0.455 ± 0.004
	8	0.899 ± 0.004
	13	1.451 ± 0.006
	20	2.24 ± 0.02
6.66	1.5	0.391 ± 0.002
	3	0.798 ± 0.007
	6	1.560 ± 0.007
	10	2.58 ± 0.03
	15	3.95 ± 0.04
6.90	2	0.886 ± 0.005
	4	1.70 ± 0.01
	8	3.36 ± 0.01
	13	5.37 ± 0.05
	20	8.32 ± 0.03
7.01	1.5	0.827 ± 0.008
	3	1.620 ± 0.007
	6	3.180 ± 20
	10	5.22 ± 0.05
	15	7.74 ± 0.14
7.16	1.5	1.170 ± 0.016
	3	2.30 ± 0.03
	6	4.44 ± 0.02
	10	6.97 ± 0.08
	15	10.35 ± 0.09

[¶] $[Cl^-] = 0.7$ M; $\lambda = 320$ nm. Errors are given as one standard deviation

Appendix VI pH dependence of observed pseudo-first-order rate constants for ascorbic acid reduction of *trans*-$[PtCl_4(NH_3)_2]$ (**11**) at 25 °C and I = 1.0 M[†]

pH	$[H_2Asc]_{tot}$/mM	k_{obsd}/s^{-1}
5.08	2.5	$(2.40 \pm 0.01) \times 10^{-2}$
	5	$(4.52 \pm 0.06) \times 10^{-2}$
	10	0.088 ± 0.002
	17	0.141 ± 0.001
	25	0.210 ± 0.002
5.47	2.5	$(4.88 \pm 0.03) \times 10^{-2}$
	5	$(9.44 \pm 0.02) \times 10^{-2}$
	10	0.189 ± 0.001
	17	0.310 ± 0.002
	25	0.466 ± 0.002
5.75	2	$(7.75 \pm 0.09) \times 10^{-2}$
	4	0.151 ± 0.001
	8	0.297 ± 0.002
	13	0.468 ± 0.003
	20	0.739 ± 0.004
6.01	1.5	0.096 ± 0.002
	3	0.192 ± 0.002
	6	0.376 ± 0.002
	10	0.615 ± 0.004
	15	0.921 ± 0.007
6.27	1.5	0.167 ± 0.003
	3	0.330 ± 0.004
	6	0.655 ± 0.003
	10	1.064 ± 0.015
	15	1.634 ± 0.013
6.51	1.5	0.273 ± 0.002
	3	0.544 ± 0.004
	6	1.100 ± 0.005
	10	1.820 ± 0.011
	15	2.70 ± 0.03

to be continued

Appendix VI continued

6.66	1.5	0.395 ± 0.003
	3	0.777 ± 0.004
	6	1.52 ± 0.01
	10	2.54 ± 0.02
	15	3.88 ± 0.04
6.87	1.5	0.606 ± 0.004
	3	1.230 ± 0.005
	6	2.39 ± 0.02
	10	3.98 ± 0.03
	15	5.85 ± 0.12
7.01	1.5	0.836 ± 0.007
	3	1.65 ± 0.01
	6	3.18 ± 0.03
	10	5.29 ± 0.06
	15	7.83 ± 0.09
7.16	1.5	1.15 ± 0.01
	3	2.23 ± 0.01
	6	4.27 ± 0.04
	7.5	5.24 ± 0.05
	15	9.68 ± 0.11

† $[Cl^-] = 0.7$ M; $\lambda = 320$ nm. Errors are given as one standard deviation

Appendix VII pH dependence of observed pseudo-first-order rate constants for ascorbic acid reduction of $[PtCl_6]^{2-}$ (**12**) at 25 °C and I = 1.0 M[§]

pH	$[H_2Asc]_{tot}$/mM	k_{obsd}/s^{-1}
5.14	2.5	$(1.55 \pm 0.01) \times 10^{-2}$
	5	$(2.93 \pm 0.02) \times 10^{-2}$
	7.5	$(4.13 \pm 0.02) \times 10^{-2}$
	12.5	$(6.52 \pm 0.05) \times 10^{-2}$
	25	0.129 ± 0.001
5.47	2.5	$(2.50 \pm 0.02) \times 10^{-2}$
	5	$(4.80 \pm 0.05) \times 10^{-2}$
	10	$(9.30 \pm 0.07) \times 10^{-2}$
	16	0.148 ± 0.002
	25	0.239 ± 0.002
5.74	1	$(1.85 \pm 0.03) \times 10^{-2}$
	2	$(3.50 \pm 0.07) \times 10^{-2}$
	4	$(6.73 \pm 0.04) \times 10^{-2}$
	7.5	0.122 ± 0.001
	15	0.245 ± 0.002
5.97	1	$(3.12 \pm 0.04) \times 10^{-2}$
	2	$(5.84 \pm 0.05) \times 10^{-2}$
	4	0.113 ± 0.002
	6.5	0.179 ± 0.002
	10	0.274 ± 0.002
6.28	7.5	0.320 ± 0.005
	7.5	$0.325 \pm 0.005^{\dagger}$
6.51	1	$(8.7 \pm 0.1) \times 10^{-2}$
	2	0.175 ± 0.002
	4	0.347 ± 0.008
	6.5	0.549 ± 0.003
	10	0.852 ± 0.007
6.68	1	0.134 ± 0.003
	2	0.267 ± 0.002

to be continued

Appendix VII continued

	4	0.521 ± 0.005
	7.5	0.96 ± 0.01
	10	1.28 ± 0.01
6.90	1	0.222 ± 0.002
	2	0.434 ± 0.002
	4	0.832 ± 0.008
	6.5	1.340 ± 0.005
	10	2.06 ± 0.01
	15	3.03 ± 0.03
7.15	1	0.385 ± 0.002
	2	0.744 ± 0.003
	4	1.43 ± 0.01
	6.5	2.26 ± 0.02
	10	3.45 ± 0.06

[§] $[Cl^-] = 0.7$ M; $\lambda = 320$ nm. Errors are given as one standard deviation

[†] Measured in the presence of $[PtCl_4]^{2-}$ (10-fold excess)

Appendix VIII pH dependence of observed pseudo-first-order rate constants for ascorbic acid reduction of $[PtBr_6]^{2-}$ (**13**) at 25 °C and $I = 1.0$ M[®]

pH	$[H_2Asc]_{tot}$/mM	k_{obsd}/s^{-1}
3.70	3.5	$(2.26 \pm 0.02) \times 10^{-2}$
	7	$(4.41 \pm 0.02) \times 10^{-2}$
	11	$(6.70 \pm 0.02) \times 10^{-2}$
	17.5	0.106 ± 0.001
	25	0.153 ± 0.001
4.10	2.5	$(3.0 \pm 0.1) \times 10^{-2}$
	5	$(5.73 \pm 0.03) \times 10^{-2}$
	7.5	$(8.6 \pm 0.2) \times 10^{-2}$

to be continued

Appendix VIII continued

	12.5	0.142 ± 0.001
	25	0.281 ± 0.002
4.35	2.5	0.035 ± 0.001
	5.0	0.073 ± 0.003
	12.5	0.184 ± 0.004
	25	0.358 ± 0.009
4.57	2	$(4.84 \pm 0.01) \times 10^{-2}$
	4	$(9.39 \pm 0.05) \times 10^{-2}$
	8	0.187 ± 0.001
	13	0.306 ± 0.002
	20	0.478 ± 0.001
4.85	2	$(7.23 \pm 0.02) \times 10^{-2}$
	4	0.147 ± 0.004
	13	0.493 ± 0.002
	20	0.770 ± 0.003
	50	1.94 ± 0.01
5.23	1	$(7.9 \pm 0.1) \times 10^{-2}$
	2	0.165 ± 0.001
	4	0.328 ± 0.003
	8	0.638 ± 0.003
	15	1.169 ± 0.009
5.74	0.5	0.104 ± 0.001
	1	0.214 ± 0.002
	2	0.424 ± 0.002
	4	0.833 ± 0.003
	7.5	1.570 ± 0.008
6.25	0.5	0.267 ± 0.001
	1	0.522 ± 0.001
	2	1.050 ± 0.003
	4	2.080 ± 0.003
	7.5	3.990 ± 0.005

to be continued

Appendix VIII continued

6.49	0.4	0.373 ± 0.002
	0.8	0.726 ± 0.002
	1.6	1.42 ± 0.01
	2.5	2.210 ± 0.004
	4	3.63 ± 0.03
6.87	0.2	0.390 ± 0.002
	0.4	0.792 ± 0.007
	0.8	1.53 ± 0.01
	1.5	2.82 ± 0.01
	2.5	4.59 ± 0.03
7.20	0.2	0.890 ± 0.007
	0.4	1.75 ± 0.01
	0.8	3.45 ± 0.02
	1.25	5.10 ± 0.02
	2.0	8.75 ± 0.04

[®] $[Br^-]$ = 0.7 M; λ = 370 - 400 nm. Errors are given as one standard deviation

Appendix IX pH dependence of observed pseudo-first-order rate constants for ascorbic acid reduction of *trans*-$[PtCl_2(en)_2]^{2+}$ **(14)** at 25 °C and I = 1.0 M[¶]

pH	$[H_2Asc]_{tot}$/mM	k_{obsd}/s^{-1}
4.27	10.0	$(2.02 \pm 0.03) \times 10^{-2}$
	17.5	$(3.46 \pm 0.02) \times 10^{-2}$
	25.0	$(5.0 \pm 0.1) \times 10^{-2}$
4.52	5.0	$(1.68 \pm 0.09) \times 10^{-2}$
	10.0	$(3.47 \pm 0.06) \times 10^{-2}$
	17.5	$(6.2 \pm 0.1) \times 10^{-2}$
	25.0	$(8.9 \pm 0.1) \times 10^{-2}$

to be continued

Appendix X pH dependence of observed pseudo-first-order rate constants for ascorbicacid reduction of *trans*-$[PtBr_2(NH_3)_4]^{2+}$ (**15**) at 25 °C and I = 1.0 M®

pH	$[H_2Asc]_{tot}$/mM	k_{obsd}/s^{-1}
2.02	5	$(4.10 \pm 0.04) \times 10^{-2}$
	10	$(7.9 \pm 0.1) \times 10^{-2}$
	15	$(11.34 \pm 0.05) \times 10^{-2}$
	25	$(18.3 \pm 0.2) \times 10^{-2}$
2.25	2	$(3.14 \pm 0.11) \times 10^{-2}$
	4	$(6.4 \pm 0.2) \times 10^{-2}$
	10	$(14.7 \pm 0.4) \times 10^{-2}$
	20	$(28.5 \pm 0.5) \times 10^{-2}$
2.50	2	$(6.56 \pm 0.11) \times 10^{-2}$
	4	$(12.4 \pm 0.3) \times 10^{-2}$
	10	$(29.3 \pm 0.3) \times 10^{-2}$
	20	$(57.6 \pm 1.9) \times 10^{-2}$
2.75	2	0.100 ± 0.003
	4	0.20 ± 0.01
	10	0.46 ± 0.01
	20	0.92 ± 0.02
3.00	2	0.174 ± 0.005
	4	0.34 ± 0.01
	10	0.78 ± 0.02
	20	1.50 ± 0.01
3.25	0.5	0.67 ± 0.002
	1.0	0.136 ± 0.003
	2.5	0.324 ± 0.012
	5.0	0.603 ± 0.021
3.50	0.5	0.105 ± 0.002
	1.0	0.200 ± 0.003
	2.5	0.442 ± 0.022
	5.0	0.866 ± 0.005
3.96	0.5	0.175 ± 0.005
	1.0	0.35 ± 0.01
	2.5	0.88 ± 0.06
	5.0	1.75 ± 0.08

® $[Br^-]$ = 0 – 50 mM, λ = 318 nm. Errors are given as one standard deviation.