MECHANISTIC STUDIES ON SUBSTITUTION REACTIONS OF SOME SQUARE PLANAR PLATINUM(II) COMPLEXES

THESIS SUBMITTED FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY IN SCIENCE
(CHEMISTRY)

OF
THE UNIVERSITY OF BURDWAN

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INDIA
MAY, 2013
Dedicated

To

My Parents
Certified that the work described in the accompanying thesis entitled, ‘**Mechanistic Studies on Substitution Reactions of Some Square Planar Platinum(II) Complexes**’ has been carried out entirely by the candidate Sri Parnajyoti Karmakar, M.Sc., under my supervision and guidance. This thesis has not been submitted previously anywhere for any degree whatsoever by him or by anyone else.

Certified further that the candidate has fulfilled all the conditions necessary for Ph.D. degree examination of the University of Burdwan.

A. K. Ghosh

(Supervisor)
Acknowledgement

First of all, I thank God Almighty for giving me the courage and ability to start and finish this thesis.

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The last, but not the least, my heartfelt gratitude goes to my father, mother and brother for their unlimited support and tolerance, and their kindest concern throughout my life.

May, 2013
Department of Chemistry
The University of Burdwan
Burdwan, West Bengal, India

Parnajyoti Karmakar
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FOREWORD

Transition metals and their reactions are in general important in the environment, in technical processes (catalysis, extraction and purification of metal complexes) and in biology and medicine (biological electron transfer, toxicology and use of metal complexes as drugs). Moreover, nonessential metal ions are very often used in biological systems either for therapeutic application or as diagnostic aids. For instance, metal complexes have been used for the treatment of many diseases (cancer, arthritis, diabetes, alzheimer’s, etc.), but with little understanding of their mechanism of action in biological systems. Biochemical studies have not clearly established the molecular basis for the activity and mechanism of action. The growing field of bioinorganic chemistry is presently dealing with the clarification of the mechanisms of action of metal complexes in biological systems. The discovery of the antitumor complex cisplatin in the late 1960s initiated extensive investigations of platinum compounds. The success of cisplatin has aroused much interest in the development of new Pt(II) complexes, such that today carboplatin and oxaliplatin are extensively used as anticancer drugs. However, many other platinum drugs have been developed to improve cisplatin. Today it is generally accepted that the antitumor activity of platinum drugs can be ascribed to interactions between the metal complex and DNA. There are many other potential biomolecules that can also react with the Pt(II) complexes, such as small molecules, proteins and enzymes. Sulfur-containing biomolecules have a high affinity for platinum. However, these interactions have been associated with negative phenomena such as nephrotoxicity, gastrointestinal toxicity and neurotoxicity. At present it is not clear how the Pt(II) species reach the DNA, because Pt(II) has a high affinity for binding to sulfur donors that compete with nitrogen donor ligands such as DNA bases. Many of the reactions with S- and N-donor nucleophiles are well investigated and understood, although the interplay of all these processes is still controversial. The objective of this work was to emanate from the simple structural composition of cisplatin and modify the properties of the complex by introducing chelating amines with different σ-donor properties instead of primary ammines. The impact of the structural changes on the reactivity of the resulting complexes was studied on the basis of their reaction with different nucleophiles. A comprehensive review of the kinetics of substitution reaction with special reference to platinum(II) complexes has been presented in the beginning of this thesis. It covers the outlines of the general mechanistic and kinetic aspects along with
different types of substitution reactions adopted by various platinum(II) metal ion systems and evaluation of the factors that affecting the substitution reaction kinetics of these complexes.

Chapter 1 describes the general introduction for inorganic reaction mechanism.

Chapter 2 deals with the kinetics and mechanism of interaction of DL-penicillamine with cis-[Pt(cis-dach)(OH₂)₂]²⁺. The kinetic studies have been done at pH 4.0 in aqueous medium. At this pH, the substrate complex exists predominantly as the diaqua species and the ligand DL-penicillamine exists mainly as zwitterion. The substitution reaction proceeds via rapid outer sphere association complex formation, followed by two slow consecutive steps. The first is the conversion of title complex into inner sphere complex and second is the slower chelation step where by another aqua ligand is replaced. The low enthalpy of activation and large negative value of entropy of activation are consistent with an associative mode of activation for both consecutive steps. (This work has been published in the journal ‘Journal of Coordination Chemistry’ vol. 63, page 2158, 2010).

Chapter 3 discusses the kinetic and mechanistic aspects of the interaction of glycyl-L-leucine (Glyleu) with cis-[Pt(cis-dach)(OH₂)₂]²⁺ ion. The reaction has been studied spectrophotometrically as a function of [cis-[Pt(cis-dach)(OH₂)₂]²⁺], [Glyleu], and temperature at pH 4.0, where the complex exists predominantly as the diaqua species and Glyleu as a zwitterion. The substitution reaction shows two consecutive steps: the first is the ligand-assisted anation and second is the chelation step. The activation parameters for both the steps were calculated using Eyring equation. The low ∆H° and large negative value of ∆S° indicate an associative mode of activation for both the aqua ligand substitution processes. (This work has been published in the journal ‘Transition Metal Chemistry’ vol. 35, page 911, 2010)

Chapter 4 comprises the mechanistic aspects of the interaction of adenosine with the same substrate complex. This reaction has also been studied at pH 4.0. The substitution reaction shows two consecutive steps: the first is the ligand-assisted anation followed by a chelation step, which is independent on the incoming ligand concentration. The activation parameters for both the steps suggest an associative mode of activation for the substitution processes. (This work has been published in the journal ‘International Journal of Chemical Kinetics’ vol. 43, page 219, 2011).
Chapter 5 illustrate the kinetics of the substitution reactions of cis-[Pt(cis-dach)(H₂O)₂]²⁺ and cis-[Pt(en)(H₂O)₂]²⁺ with excess N,N'-diethylthiourea in aqueous solution. The effect of different N-N spectator ligand on the reactivity of platinum(II) complexes was investigated by studying the water lability of the reactant complexes. The reactions follow normal square-planar substitution mainly in an associative way. Rate parameters have been evaluated under different conditions. At a particular pH 3.0, the substrate complex exists predominantly as the diaqua species and the ligand as mixture of protonated and neutral molecule. The ligand acts as monodentate sulphur donor ligand. The reaction proceeds via two consecutive steps, where both the steps are ligand dependent. Activation parameters have been calculated using Eyring equation. The low $\Delta H^\neq$ and large negative values of $\Delta S^\neq$ as well as $\Delta H^\neq$ and $\Delta S^\neq$ indicate an associative mode of activation for both aqua molecule substitution processes. (This work has been published in the journal ‘Journal of Solution Chemistry’ vol. 42, page 441, 2013).

Chapter 6 demonstrate the kinetics of interaction of cis-[Pt(cis-dach)(H₂O)₂]²⁺ with three different amino acids, namely L-Asparagine, L-Arginine and L-Glutamic acid. The substitution of both coordinated water molecules have been investigated under pseudo-first-order conditions as a function of concentration of the reactants, temperature and pH at constant ionic strength. The interaction reaction observes two subsequent reaction steps: the first step is the ligand-assisted anation and the second one is the chelation step. The rate constants for the first step increase with increasing amino acid concentration and the calculated activation parameters for all reactions suggest an associative substitution mechanism for both the aqua ligand substitution processes. (This work has been accepted in the journal ‘Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry’).

Chapter 7 portray the substitution reaction of cis-[Pt(cis-dach)(H₂O)₂]²⁺ with three ligands of different donicity, namely 2-thiouracil (S, N), 1,2-cyclohexanedionedioxime (N, N) and acetylacetone (O, O). The substitution reaction proceeds via rapid outer sphere association complex formation, followed by two slow consecutive steps. The first of these involves ligand-assisted deaquation, while second involves chelation as the second aqua ligand is displaced. The association equilibrium constant ($K_E$) for the outer sphere complex formation has been evaluated together with rate constants for the two subsequent steps. The rate constants for the first step increase with increasing ligand concentration and the evaluated activation parameters for all
reactions suggest an associative substitution mechanism for both the aqua ligand substitution processes. (This work has been accepted in the journal ‘Journal of Chemical Sciences’).

**Chapter 8** the last chapter deals with discussions based on all the experimental facts. These types of interaction studies are important, not only to help in the design of future platinum-based drugs, but also from a bioinorganic viewpoint in order to gain further understanding of other important reactions occurring in the body.
Abbreviations Used

Ligands

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad</td>
<td>adenosine</td>
</tr>
<tr>
<td>amp</td>
<td>aminomethylpyridine</td>
</tr>
<tr>
<td>bpy</td>
<td>2,2'-bipyridine</td>
</tr>
<tr>
<td>dach</td>
<td>1,2-cis-R,S-diaminocyclohexane</td>
</tr>
<tr>
<td>detu</td>
<td>N,N'-diethylthiourea</td>
</tr>
<tr>
<td>en</td>
<td>ethylenediamine</td>
</tr>
<tr>
<td>Glyleu</td>
<td>glycyl-L-leucine</td>
</tr>
</tbody>
</table>

Complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>cis-[Pt(NH$_3$)$_2$Cl$_2$]</td>
</tr>
<tr>
<td>DDP</td>
<td>diamminedichloroplatinum(II)</td>
</tr>
<tr>
<td>Pt(amp)</td>
<td>cis-[Pt(aminomethylpyridine)(H$_2$O)$_2$]$^{2+}$</td>
</tr>
<tr>
<td>Pt(bpy)</td>
<td>cis-[Pt(N,N'-bipyridine)(H$_2$O)$_2$]$^{2+}$</td>
</tr>
<tr>
<td>Pt(dach)</td>
<td>cis-[Pt(1,2-cis-R,S-diaminocyclohexane)(H$_2$O)$_2$]$^{2+}$</td>
</tr>
<tr>
<td>Pt(en)</td>
<td>cis-[Pt(ethylenediamine)(H$_2$O)$_2$]$^{2+}$</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>[Pt(1,2-trans-R,R-diaminocyclohexane)(O-O-oxalato)]</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>[Pt(NH$_3$)$_2$(O,O-cyclobutane-1,1-dicarboxylate)]</td>
</tr>
<tr>
<td>AMD473</td>
<td>cis-[Pt(NH$_3$)(2-picoline)Cl$_2$]</td>
</tr>
</tbody>
</table>

Other Chemicals

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>5′GMP</td>
<td>guanosine-5′-monophosphate</td>
</tr>
<tr>
<td>GSH</td>
<td>γ-glutamylcysteinylglycine (glutathione)</td>
</tr>
<tr>
<td>tu</td>
<td>thiourea</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulphoxide</td>
</tr>
<tr>
<td>py</td>
<td>pyridine</td>
</tr>
</tbody>
</table>

Other Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>R</td>
<td>gas constant, 8.3145 J K$^{-1}$ mol$^{-1}$</td>
</tr>
</tbody>
</table>
\( h \)  Planck constant, \( 6.6261 \times 10^{-34} \text{ J s} \)

\( k_B \)  Boltzmann constant, \( 1.3807 \times 10^{-23} \text{ J K}^{-1} \)

\( k_{\text{obs}} \)  observed rate constant for pseudo-first-order reactions

\( k_1, k_2 \)  rate constants

\( K \)  equilibrium constant

\( T \)  temperature

\( I \)  ionic strength

\( \text{TS} \)  transition state

\( X \)  leaving group: \( \text{H}_2\text{O} \)

\( \text{DFT} \)  density functional theory

\( \text{NMR} \)  nuclear magnetic resonance

\( \text{ESI-MS} \)  electron spray ionization mass spectrometry

\( \text{IR} \)  infrared

\( \Delta H^\# \)  activation enthalpy

\( \Delta S^\# \)  activation entropy
Chapter 1
General Introduction

Section I: A brief survey on kinetic and mechanistic aspects of ligand substitution reactions on square planar platinum(II) complexes.

Section II: Some recent studies on ligand substitution reactions of platinum(II) complexes.

Section III: Biological importance of platinum–am(m)ine chemistry in reference to antitumour activity.

Section IV: Objectives and importance of the present work.

Section V: Instrumental techniques and general information about the chemicals.
Section I
A brief survey on kinetic and mechanistic aspects of ligand substitution reactions on square planar platinum(II) complexes

1. (I) 1 Introduction

The most fundamental reaction a complex can undergo is ligand substitution reaction, a reaction in which one Lewis base displaces another from a Lewis acid:

\[
MX_n + Y \rightleftharpoons MX_{n-1}Y + X
\]  

(1.1)

This class of reaction includes complex formation, in which the leaving group, the displaced base X and the entering group, the displacing base Y, is some other ligand (one of the ligands involved is often also the solvent species). The rates of such reactions vary widely, ranging from completion within the time for reactant mixing to years. H. Taube called complexes having substitution half life \( t_{1/2} < 30 \) sec as labile and called those with longer \( t_{1/2} \) as inert [1]. Studying reactions of labile complexes require techniques such as stopped-flow [2], P-jump [3], or T-jump [4] (in which system at equilibrium is perturbed by a sudden change in pressure or temperature and its relaxation to a new equilibrium monitored)

Slow reactions can be monitored by conventional techniques (including NMR, UV-Vis spectroscopy, and polarimetry). Hence, considerably more information is available on reactions of inert species.

After the appearance of landmark review on ligand substitution dynamics by Taube, there has been extensive growth in the field of reaction mechanism [1]. While many of the kinetic problems have been answered, newer questions have been raised by these answers and much work has yet to be done to reconcile all the facts on a sound theoretical basis. Sophisticated instruments are now in continuous use to discover the intimate mechanism. Several important treatise and reviews have been appeared to enlighten the progress achieved [5-16].
1. Classification of ligand substitution reactions

Ligand substitution reactions of coordination complexes can be illustrated by the general equation,

$$MX_n + Y \rightarrow MX_{n-1}Y + X$$ (1.2)

where ‘M’ is a metal atom or ion and ‘X’, ‘Y’ are any two ligands. For simplicity the charges have been ignored. In keeping with organic chemistry terminology, substitution reactions have been conveniently divided into nucleophilic (SN) and electrophilic (SE) substitutions:

$$MX_n + M^+ \rightarrow M^+X_n + M$$ \hspace{1cm} (1.3) \hspace{1cm} S_E

Electrophilic substitution mechanisms will not be considered further. For a ligand substitution process, S_N mechanisms are relevant and can be further subdivided into two paths like:

(i) S_N1 dissociation (substitution, nucleophilic, unimolecular):

This type of reaction can be illustrated as:

$$MX_n \underset{\text{slow}}{\xrightarrow{\text{fast}}} MX_{n-1} + X$$ (1.5)

$$MX_{n-1} + Y \rightarrow MX_{n-1}Y$$ (1.6)

Such reactions are insensitive to the nature of the incoming nucleophile, ‘Y’, but sensitive to the leaving group ‘X’ and reach the transition state principally by the internal accumulation of the energy to break the bond to the leaving group. The detection of an intermediate of reduced coordination number is the best diagnosis of the S_N1 mechanism.

(ii) S_N2 displacement (substitution, nucleophilic, bimolecular):

This type of reaction involves a bimolecular rate determining step followed by a rapid cleavage of ‘X’

$$MX_n + Y \underset{\text{slow}}{\xrightarrow{\text{fast}}} MX_{n-1}Y + X$$ (1.7)

These reactions are affected by the nature of the entering group. Stereospecificity i.e. retention of configuration suggests an S_N2 reaction.

One of the great complicating factors in assigning mechanism of substitution reactions is the existence of borderline mechanism or intermediate mechanism between S_N1 and S_N2. Depending upon the nature of participation of entering ligand in the
transition state, it has been suggested to classify [17] ligand substitution reactions into four categories:
(a) $S_{N1}(\text{lim})$, where the rate determining step involves only bond breaking and definite evidence for intermediate with reduced coordination number exists;
(b) $S_{N1}$, in which bond breaking is important in the rate determining step but no evidence for the existence of intermediate of reduced coordination can be presented;
(c) $S_{N2}(\text{lim})$, in which the rate determining step involves only ligand-substrate bond making and definite evidence for intermediate of increased coordination number exists;
(d) $S_{N2}$, in which the rate determining step involves about equal bond making and bond breaking in the transition state.

An alternative classification has been proposed by Langford and Stengle [7]. According to them the ligand substitution processes can be classified in terms of stoichiometric and intimate mechanism. Stoichiometric mechanism relates to the identification of the sequence of elementary steps involved in a complicated overall reaction. Intimate mechanism is the understanding of the magnitude of the rate constants for the individual steps in terms of rearrangements of atoms and bonds.

Stoichiometric Mechanism: Three paths for ligand substitutions are illustrated below:

$$
\begin{align*}
\text{MX}_n + X & \rightarrow \text{MX}_{n-1} + Y \rightarrow \text{MX}_{n-1} Y \quad (\text{D}-\text{path}) \\
\text{MX}_n + Y & \rightarrow \text{MX}_n Y \leftarrow X \rightarrow \text{MX}_{n-1} Y \quad (\text{A}-\text{path}) \\
\text{MX}_n \ldots Y & \rightarrow \text{MX}_{n-1} Y \ldots X \quad (\text{I}-\text{path})
\end{align*}
$$

In dissociative path (D), the leaving ligand is lost in the first step, producing an intermediate of reduced coordination number. In associative (A) path the entering ligand adds to the complex in the first step, producing an intermediate of increased coordination number. In the concerted path termed interchange (I), the leaving group is moving from the inner to outer sphere while the entering group is moving from outer to inner sphere.
Intimate mechanism: Two categories of intimate mechanism may be distinguished operationally.

1. Associative activation (a): The reaction rate is approximately as sensitive to variation of the entering group as to variation of the leaving group.

2. Dissociative activation (d): The reaction rate is much more sensitive to variation of the leaving group than to the variation of the entering group.

D mechanism must be dissociative. A mechanism must be associative. ‘I’ reaction may have a variety of transition states, but two well-defined types will be those resembling the transition state of A and D reactions. The A-like transition in an ‘I’ process will display substantial bonding to both the entering and leaving groups, and the entering group will display an important part in determining its energy. Such a transition state in an I path will be indicated by adding a notation ‘a’ (i.e. Iₐ). The D-like transition state of I reaction is one with only weak bonding to both the entering and leaving groups (the bonding may be very weak indeed). The entering group effect on the reaction rate will be small. Such an ‘I’ process will be denoted with an added d (i.e. Iₐ). However, the effect of charge and size of the central metal ion influences the mechanism of a reaction. This can be summarized in Table 1.1.

**Table 1.1:** Effect of size and charge on the rate of reactions involving dissociative (D), interchange (I) and associative (A) mechanism:

<table>
<thead>
<tr>
<th>Changes</th>
<th>Effects on rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D process</td>
</tr>
<tr>
<td>Increase in positive charge of central atom</td>
<td>Decrease</td>
</tr>
<tr>
<td>Increase in size of central atom</td>
<td>Increase</td>
</tr>
<tr>
<td>Increase in negative charge of entering group</td>
<td>No effect</td>
</tr>
<tr>
<td>Increase in size of entering group</td>
<td>No effect</td>
</tr>
<tr>
<td>Increase in negative charge of leaving group</td>
<td>Decrease</td>
</tr>
<tr>
<td>Increase in size of leaving group</td>
<td>Increase</td>
</tr>
<tr>
<td>Increase in negative charge of non-replaceable group</td>
<td>Increase</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Increase in size of non-replaceable group</td>
<td>Increase</td>
</tr>
</tbody>
</table>

These processes can be represented as below:

Scheme 1.1

In this scheme, the large circle represents the total coordination sphere of the metal ion and the small circle represents an (identical) entering and leaving ligand molecule respectively.

Following Basolo and Pearson [5], a correlation can be drawn between Langford’s mechanistic designation and Hughes-Ingold’s classification. The A-path of ligand substitution processes corresponds to $S_N 2(lim)$. The D-path corresponds to $S_N 1(lim)$. The I$_a$ mechanism would be $S_N 2$ path and the I$_d$ mechanism parallel to $S_N 1$
process. IUPAC recommendation for the representation of reaction mechanisms is summarized in Table 1.2.

The rate law of ligand substitution reaction by an interchange mechanism is also consistent with an Eigen-Wilkins mechanism in which an encounter complex is formed in the pre-equilibrium steps [11]. The diffusion controlled encounter complex between MX$_n$ and the entering group diffuse together and come into contact. They may also separate at diffusion limited rates. The next step is the rate determining reaction of the encounter complex to give the final products. This can be represented by Scheme 1.2:

\[
\begin{align*}
X_nM + Y & \xrightarrow{K_E, \text{ fast}} X_nM \ldots Y \\
X_nM\ldots\ldots Y & \xrightarrow{k_1 \text{ r/d step}} X_{n-1}MY \ldots X \\
X_{n-1}MY \ldots X & \xrightarrow{\text{fast}} X_{n-1}MY + X
\end{align*}
\]

**Scheme 1.2**

The rate of the reaction = $k_1[X_nM \ldots Y] = k_1K_E[MX_n][Y]$

If, $[MX_n]_{\text{total}} = [MX_n] + \{X_nM \ldots Y\}$

then, $[MX_n]_{\text{total}} = [MX_n] + K_E[MX_n][Y]$;

and $[MX_n] = \{[MX_n]_{\text{total}}/(1 + K_E[Y])\}$

Rate = $\{k_1K_E[MX_n]_{\text{total}}[Y]/(1 + K_E[Y])\}$ (1.11)

and $k_{\text{obs}} = \{k_1K_E[Y]/(1 + K_E[Y])\}$

This equation holds for the reactions, which involve outer sphere association.

**Table 1.2: Comparison of Ingold System Names with IUPAC Recommendation for the Representation of Reaction Mechanisms**

<table>
<thead>
<tr>
<th>Ingold System Name</th>
<th>IUPAC Proposed Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SUBSTITUTION MECHANISMS</strong></td>
<td></td>
</tr>
<tr>
<td>$S_{N2}$</td>
<td>$A_ND_N$</td>
</tr>
<tr>
<td>$S_{E2}$</td>
<td>$D_EA_E$</td>
</tr>
<tr>
<td>$S_{E2}$ or $S_{E2}$ coord</td>
<td>$A_n + $ cyclo-$D_EA_ED_n$</td>
</tr>
<tr>
<td>$A_{AC2}$</td>
<td>$A_h + A_N + A_hD_h + D_N + D_h$</td>
</tr>
<tr>
<td>$S_{N1}$ or $B_{AL1}$</td>
<td>$D_N + A_N$</td>
</tr>
<tr>
<td>$S_{Ni}$</td>
<td>$D_N + D + A_N$</td>
</tr>
</tbody>
</table>
1. (I) 3 Substitution reactions in square planar complexes

1. (I) 3a General discussion

Metal ions with the d⁸ configuration [Au(III), Pt(II), Pd(II), Rh(I) and Ir(I)] usually form four coordinated square planar complexes, especially with strong field ligands. Ligand substitution reactions of square planar geometry are mainly confined to platinum(II) because the reaction occurs on a timescale that is very amenable to study. More recently, the availability of methods [18] for following faster reactions has opened the area to study the highly labile palladium(II) and other d⁸ systems.

Ligand substitution processes of Pt(II) complexes appear to proceed generally by associative mechanism. This is due to lack of steric crowding and availability of an empty ‘p’ orbital perpendicular to the molecular plane. In some cases, however, steric requirements demand dissociative path [19, 20]. Another recognized pathway is oxidative addition followed by reductive elimination leading to ligand substitution [21].

Extensive studies on the ligand substitution of Pt(II) and Pd(II) complexes have shown that:
(a) sterically uncrowded four coordinated systems have a definite tendency to form five and six coordinated species;
(b) rates of these reactions are dependent on the nature and concentrations of the incoming ligands;
(c) steric effects, i.e. trans effect and cis effect play an important role in reaction kinetics.

However, Pd(II) complexes differ from Pt(II) complexes in that the trans effect is insignificant in the former systems.

1. (i) 3b Stoichiometric Mechanism

\[ \text{L}_3\text{MX} + \text{Y} \rightarrow \text{L}_3\text{MY} + \text{X} \]  \hspace{1cm} (1.12)

The kinetics of the above type of reaction follows a two term rate law and can be described by rate equation

\[ \text{Rate} = k_{\text{obs}} [\text{L}_3\text{MX}] \]

where, \( k_{\text{obs}} = k_1 + k_2[Y] \).

\( k_1 \) and \( k_2 \) are first and second order rate constants respectively, \([Y]\) is the concentration of the entering ligand. The two term rate law indicates that the product formation takes place via two parallel reaction paths. These reactions are generally studied under pseudo first order conditions. The rate is measured at several values of \([Y]\) and the rate constant \( k_{\text{obs}} \) is plotted against \([Y]\). The plot is linear in nature with an intercept. The intercept of the plot gives the value of \( k_1 \), while that of \( k_2 \) is obtained from the slope. The values of \( k_2 \) differ for different nucleophiles \( Y \), but the value of \( k_1 \) remains unchanged for all nucleophiles except for the solvent present in the reaction. Hence the \( k_1 \) term originates due to nucleophilic attack of the solvent and \( k_2 \) term originates due to nucleophilic attack by \( Y \). \( k_1 \) is related to solvent path and \( k_2 \) is related to nucleophilic path. The stoichiometric mechanisms involved in different nucleophilic substitutions concerning either solvent or nucleophilic or both paths are different and present different rate expressions as different solvent paths are involved.

For the reaction, \( \text{L}_3\text{MX} + \text{Y} \rightarrow \text{L}_3\text{MY} + \text{X} \), the possible stoichiometric mechanisms are discussed below. For irreversible solvolysis the Scheme 1.3 can be used:

\[ \text{Schem} 1.3 \]

\[ \text{Rate} = \frac{d[\text{ML}_3\text{Y}]}{dt} = \frac{d[\text{ML}_3\text{XS}]}{dt} + \frac{d[\text{ML}_3\text{XY}]}{dt} \]

\[ = k_8[S][\text{ML}_3\text{X}] + k_2[Y][\text{ML}_3\text{X}] \]
\[ \begin{align*}
&= (k_S[S] + k_2[Y])[ML_3X] \\
&= (k_1 + k_2[Y])[ML_2X] \quad \text{where } k_S[S] = k_1 \\
&= k_{obs}[ML_3X] \quad \text{where } k_{obs} = k_1 + k_2[Y]
\end{align*} \]

According to the Scheme 1.2, the substrate may undergo rate determining associative attack either by incoming ligand Y, or by a molecule of solvent S, in steps governed by the rate constants \(k_2\) and \(k_s\) respectively. The five coordinated species thus formed (which may be active intermediate or transition state) subsequently decay to products. With weak nucleophiles and polar solvents, the reaction is dominated by the solvent path i.e. the value of \(k_2\) is small or zero. The kinetics of the reaction between \([Pt(dien)Br]^+\) and the nucleophiles \(N_3^-, NO_2^-, py, Cl^-\) and \(OH^-\) in aqueous solution justify this [22]. The exchange reaction (1.14) was carried out in solvents hexane and methanol [23].

\[
Pr_3PPrCl_2NHEt_2 + *NHEt_2 \longrightarrow Pr_3PPrCl_2*NHEt_2 + NHEt_2
\]

In hexane \(k_1\) is zero while in methanol \(k_2\) is zero. Solvents, which are poorly coordinating in nature, cannot afford the solvent path and the nucleophile or ligand path dominates. Hexane is a poor coordinating solvent, so \(k_1\) is zero.

For reversible solvolysis with highly reactive solvent substituted intermediate, the reaction Scheme 1.4 can be proposed [24].

\[
\begin{align*}
&L_3M X \xrightarrow{k_{Ss} + Ss - X} L_3M S \xrightarrow{k_{Ee} + Y, - S} L_3M Y \\
&k_2, + Y, - X
\end{align*}
\]

**Scheme 1.4**

Rate = \(d[L_3MY]/dt = k_f [L_3MS][Y] + k_2[L_3MX][Y]\)

Applying steady state approximation for the solvent substituted intermediate \(L_3MS\), the following relation is obtained.

\[
d[L_3MS]/dt = 0
\]

i.e., \(k_S[S][L_3MX] - k_{Ss}[L_3MS][X] - k_f[L_3MS][Y] = 0\)

or, \([L_3MS] = \{k_S[S]/(k_{Ss}[X] + k_f[Y])\}[L_3MX]\)

Rate = \(\{k_f k_S[S][Y][L_3MX] / (k_{Ss}[X] + k_f[Y])\} + k_2[L_3MX][Y]\) \hspace{1cm} (1.15)

\(k_{obs} = \{k_f k_1[Y] / (k_{Ss}[X] + k_f[Y])\} + k_2[Y], \text{ since } k_S[S] = k_1\)
Inosine, a nucleoside derivative, replaces $\text{Br}^-$ ligand in $[\text{Pt(dien)}\text{Br}]^+$ corresponding to above scheme [25].

$$[\text{Pt(dien)}\text{Br}]^+ + \text{Ino} \rightarrow [\text{Pt(dien)}(\text{Ino})]^2^+ + \text{Br}^-$$

$$k_{\text{obs}} = \frac{k_1k_f[\text{Ino}]}{k_f[\text{Ino}] + k_2[\text{Br}^-]} + k_2[\text{Ino}] \quad (1.16)$$

The $k_2[\text{Ino}]$ term represents the ligand path and the complex term corresponds to the solvolytic path. The reaction mainly proceeds through the solvolytic path and is inhibited by $\text{Br}^-$. The leaving group also competes effectively with the entering group for the solvent substituted intermediate in the reaction between oxalate ion and cisplatin [26], $\text{cis-}[\text{Pt(NH}_3)_2\text{Cl}_2]$. This reaction is governed by the Scheme 1.5.

$$\text{cis-}[\text{PtCl}_2(\text{NH}_3)_2] + \text{H}_2\text{O} \quad \stackrel{k_s, - \text{Cl}^-, + \text{H}_2\text{O}}{\longrightarrow} \quad \text{cis-}[\text{PtCl}(\text{H}_2\text{O})(\text{NH}_3)_2]^+ + \text{Cl}^-$$

$$\quad \stackrel{k_f, + \text{Ox}^{2^-}}{\longrightarrow} \quad \text{cis-}[\text{Pt(Ox)(NH}_3)_2]$$

**Scheme 1.5**

The rate of the reaction = $k_f[\text{cis-PtCl(H}_2\text{O)(NH}_3)_2]^+[\text{Ox}^{2^-}]$

Applying steady state approximation for the mono aqua complex,

$$k_s[\text{PtCl}_2(\text{NH}_3)_2][\text{H}_2\text{O}] - k_s[\text{PtCl(H}_2\text{O})(\text{NH}_3)_2]^+[\text{Cl}^-] - k_f[\text{PtCl(H}_2\text{O})(\text{NH}_3)_2]^+[\text{Ox}^{2^-}] = 0$$

$$[\text{PtCl(H}_2\text{O})(\text{NH}_3)_2]^+ = k_s[\text{PtCl}_2(\text{NH}_3)_2][\text{H}_2\text{O}]/(k_s[\text{Cl}^-] + k_f[\text{Ox}^{2^-}])$$

Rate = $\{k_fk_s[\text{H}_2\text{O}] / (k_s[\text{Cl}^-] + k_f[\text{Ox}^{2^-}])\}[\text{PtCl}_2(\text{NH}_3)_2]$ \hspace{1cm} (1.17)

$$k_{\text{obs}} = \{k_fk_s[\text{H}_2\text{O}] / (k_s[\text{Cl}^-] + k_f[\text{Ox}^{2^-}])\}$$

In this reaction the leaving group $\text{Cl}^-$ competes with $\text{Ox}^{2^-}$ for mono aqua complex and retards the rate.

When the reaction of the solvent substituted complex with the incoming ligand is slow compared to the rate of attainment of solvolysis equilibrium i.e. when the solvolysis
is in a rapidly attained pre-equilibrium, the rate is determined by the interaction of incoming ligand Y and each of the complexes ML₃X and ML₃S (Scheme 1.6).

\[ \text{L₃M X} + S \xrightarrow{K_S, \text{fast}} \text{ML₃S} + X \]
\[ \xrightarrow{k_1, + Y, - S} \text{ML₃Y} \]

**Scheme 1.6**

If, \([\text{ML₃X}]_0\) be the initial concentration of \(\text{ML₃X}\), then,

\[ [\text{ML₃X}]_0 = [\text{ML₃X}] + [\text{ML₃S}] \]

Again the equilibrium constant \(K_S\) is defined as:

\[ K_S = \{[[\text{ML₃S}][X]]/[\text{ML₃X}] \]

or, \([\text{ML₃S}] = K_S[\text{ML₃X}]/[X] \]

Now,

\[ [\text{ML₃X}]_0 = ([\text{ML₃X}] + K_S[\text{ML₃X}])/[X] \]
\[ = (K_S + [X])[\text{ML₃X}]/[X] \]

or,

\[ [\text{ML₃X}] = \{[[X]/(K_S + [X])][\text{ML₃X}]_0 \]

Now, the rate = \(d[\text{ML₃Y}]/dt\)

\[ = \{(k_1K_S[Y])/(K_S + [X]) + (k_2[X][Y])/(K_S + [X])\}[\text{ML₃X}]_0 \quad (1.18) \]

Since, \([\text{ML₃S}] = K_S[\text{ML₃X}]_0 / (K_S + [X]) \)

\[ = \{(k_1K_S+k_2[X])/(K_S + [X])\}[Y][\text{ML₃X}]_0 \]

\(k_{obs} = \{(k_1K_S + k_2[X])/(K_S + [X])\}[Y] \)

The rate law has been proposed for the reactions of PtCl₄²⁻ and PdCl₄²⁻ with dipyridyl and ortho-phenanthroline [27-31].

1. (I) 3c Intimate Mechanism

The bulk of the evidence indicates that the substitution reactions of square planar complexes are associative in nature (A or at least Iₐ). In associative activation, the incoming ligand ‘Y’ attack the metal either from below or from above the square plane leading to a five coordinated species, which rapidly transforms to trigonal bipyramidal species. From steric considerations, the geometry of the transition state is expected to be trigonal bipyramidal, since this arrangement minimizes mutual repulsions of the five ligands. Moreover, substitution with steric retention can be explained on the basis of the
model. The leaving group ‘X’, the spectator ligand ‘T’, trans to ‘X’ and the entering ligand ‘Y’ form the triangular plane while the cis ligands lie on the perpendicular C₃ axis. The steric course of the reaction is represented below (Scheme 1.7).

![Scheme 1.7](image)

Intimate analysis of the five coordinated transition state shows that the three ligands that occupy the trigonal plane in the trigonal bipyramid may take advantage of certain σ- and π-bonding possibilities which are substantially changed from the ground state square planar complex. On the other hand, the bonding situation of the two apical ligands is not appreciably different from the ground state complex. Thus, the assumption of approximate trigonal bipyramidal geometry for the transition state correctly predicts relatively large substituent effects for the ligands in the trigonal plane (the trans, entering and leaving groups) and relatively small effects for the cis groups. NMR spectroscopic measurements show that five coordinated [Pd(Br)₂(PMe₃)₃] and [Pd(Br)(PMe₃)₄]⁡ readily lose Br⁻ and PMe₃ respectively in CD₂Cl₂ solution [32]. Several examples of four and five coordination equilibria of reaction (1.19) have been detected by variable temperature NMR spectroscopy [33].

$$[\text{PtX}_2(\text{PR}_3)_2] + \text{PR}_3 \rightleftharpoons [\text{PtX}_2(\text{PR}_3)_3] \quad (1.19)$$

Clearly, then, having formed a five coordinated species, the loss of a ligand is the necessarily next step, even when the five coordinated complexes are stable enough to be isolated and examined.
The solvent exchange at the solvent coordinated-ion e.g., the exchange of free and coordinated water at [Pt(H₂O)₄]²⁺ has been followed [34, 35] at various temperatures and pressures by using ¹⁷O NMR spectroscopy with enriched H₂¹⁷O. Negative values of ΔS^≠ and ΔV^≠ indicate A or Iₐ activation processes of water exchange.

1. (I) 4 Factors affecting the rates of substitution reaction in square planar complexes

1. (I) 4a Effect of ligands

1. (I) 4aa Trans Effect

The spectator ligands that are trans to leaving group in square planar complexes influence the rate of substitution. This phenomenon is called trans effect. The trans effect was first recognized by Werner and elaborated on by Chernyayev (1926), is very important for better understanding of the kinetic behavior of the planar complexes in particular. It is generally accepted that the trans effect arises from two separate influences: one arising in the ground state and the other in the transition state itself. Of all the specific ligand effects in substitution reactions, the trans effect is probably the most dramatic, spanning several orders of magnitude in rate (~10⁵). The effect of the trans ligand ‘L’ on the rate of the reactions (1.20) in ethanol was studied by Basolo and others [36].

trans-[Pt(PEt₃)₂(L)Cl] + Py ⇌ trans-[Pt(PEt₃)₂(L)Py]⁺ + Cl⁻ (1.20)

Although trans effect order of ligands is undoubtedly dependent on the substrate, an average order of trans labilisation is [37]:

CO, CN⁻, C₂H₄ > PR₃, H⁻ > CH₃⁻, SC(NH₂)₂ > C₆H₅⁻, NO₂⁻, I⁻, SCN⁻ > Br⁻, Cl⁻ > Py, NH₃, OH⁻, H₂O.

This order is approximately the order of increasing overlap of ligand orbitals with either a σ-Pt(6p) or π-Pt(5d) orbital. Greater the overlap, stronger is the trans effect. Depending on the nature of overlap, trans effect can be divided in two classes: σ-trans effect and π-trans effect. σ-trans effect is due to overlap of appropriate ligand pσ valence orbital with a Pt(6pσ) orbital. Thus, when the trans group is a strong σ-donor ligand, the approximate increasing σ-trans effect order is:

OH⁻ < NH₃ < Cl⁻ < Br⁻ < CN⁻, CO, CH₃ < I⁻ < SCN⁻ < PR₃ < H⁻.
Similarly, \( \pi \)-trans effect is due to overlap between ligand \( \pi^* \) orbital and Pt(5d\( \pi \)) orbital and the increasing order is:

\[
\text{Br}^- < \Gamma^- < \text{NCS}^- < \text{NO}_2^- < \text{CN}^- < \text{CO}, \text{C}_2\text{H}_4.
\]

Generally, a group with greater trans effect is replaced least easily and acts as a powerful nucleophile. This is expected for a trigonal bipyramidal transition state since the trans group and the entering group both are in similar positions with respect to the leaving group in the trigonal plane.

1. (I) 4ab Cis Effect

Studies have also been made for a possible cis effect in square planar substitution, that is, labialization of a group cis to a particular group. In contrast to the profound influence of the trans-group on the rate of substitution, cis-groups have only a very small effect. Tucker and coworkers [38] have shown that ‘cis effect’ can be more important than ‘trans effect’ when groups of nearly equal trans effect are compared. Thus in the substitution reactions of chloroammine Pt(II) complexes, it was found that the difference in the cis effects of NH\(_3\) and Cl\(^-\) (NH\(_3\) > Cl\(^-\)) is larger than their trans effect difference (Cl\(^-\) > NH\(_3\)). Therefore, the cis ligands determine the relative rates in these complexes. The kinetic studies reported by Elding and Groning [39] on the replacement of H\(_2\)O by Cl\(^-\) in a Pt(II) complex led to elaborate the effect of cis ligand on the leaving group. In the reaction (1.21), the activated complex of trigonal bipyramid

\[
\text{cis-[Pt(DMSO)Cl(H}_2\text{O)\text{]}^+ + \text{Cl}^- \rightleftharpoons \text{cis-[Pt(DMSO)Cl\text{}_{2}(H}_2\text{O)\text{]} + H}_2\text{O}} \tag{1.21}
\]

geometry with two cis ligands at the apices of trigonal bipyramid will be much stronger dipole because one of them is H\(_2\)O and the other is halide. The forward reaction should therefore be accelerated in a polar solvent due to better solvation of the transition state. The difference in rate is mainly due to the variation of the entropy of activation (\(\Delta S^\circ\)) caused by differences in solvation between the reactant ion and the activated complex.

The cis effect series in the decreasing order is:

\[
\text{DMSO} > \Gamma^- > \text{H}_2\text{O} \sim \text{NH}_3 > \text{Cl}^- > \text{Br}^- > \text{C}_2\text{H}_4
\]

Grinberg [40] has pointed out that pyridine substitutes a little faster in [Pt(py)Cl\(_3\)]\(^-\) than in [Pt(NH\(_3\))Cl\(_3\)]\(^-\). There is also some evidence [41] that Cl\(^-\) is replaced from Pt(II) complexes when cis to NH\(_3\) about 1.2 times faster than when cis to NO\(_2\)\(^-\). This gives a cis effect order: py > NH\(_3\) > NO\(_2\)\(^-\) for the substrates involved.
1. (I) 4ac Effect of the incoming group

In an associative mechanism, the incoming group is an important factor in determining the rate of a reaction. The size of the rate constant, $k_2$, is a measure of the effectiveness of the incoming group. The quantitative measure of the effectiveness or reactivity of the incoming group is termed nucleophilicity. Comparing the rates of reaction of a series of complexes with different entering groups and the same leaving group e.g. the reaction (1.22) was carried out in aqueous solution at 25°C establishes the above generalization.

$$k_2 \quad \text{Pt(dien)Br}^+ + Y^- \rightarrow \text{Pt(dien) Y}^+ + Br^- \quad (1.22)$$

The entering ligands may be arranged in decreasing order of the $k_2$ values as:

$$\text{SC(NH}_2\text{)}_2 > \text{SCN}^- > \Gamma > \text{N}_3^- > \text{NO}_2^- > \text{py} > \text{Cl}^- > \text{OH}^-$$

A thorough study of reactions of different ligands especially with trans-Pt(py)$_2$Cl$_2$ in methanol solution has been reported [42]. The reactivity order is: $\text{S}_2\text{O}_3^{2-} > \text{SC(NH}_2\text{)}_2$, $\text{C}_6\text{H}_5\text{S}^- > \text{SeCN}^- >> \text{SO}_3^{2-} > \text{SCN}^- > \Gamma >> \text{C}_6\text{H}_5\text{SH} > \text{Br}^- > \text{N}_2\text{H}_4, \text{NH}_2\text{OH} > \text{N}_3^- > \text{NO}_2^- > \text{py} > \text{NH}_3 > \text{Cl}^- > \text{MeO}^-$.

1. (I) 4ad Nucleophilicity parameter

The effect of entering group on the rate can be exemplified by reaction (1.23) carried in methanol [42, 43]:

$$\text{trans-[Pt(py)_2Cl_2]} + Y \quad \rightarrow \quad k_2 \quad \text{trans-[Pt(py)_2ClY]}^+ + \text{Cl}^- \quad (1.23)$$

Values of $k_2$ provide a measure of nucleophilicity towards trans-[Pt(py)$_2$Cl$_2$]. The nature of ‘$Y$’ affects the rate over a large range $\sim 10^9$ in the series of reactions where $Y = \text{NH}_3, \text{pyridine}, \text{Cl}^-, \text{Br}^-, \Gamma, \text{SCN}^-, \text{thiourea}$ etc. This large range of reactivity is a feature of associative mechanism of Pt(II) complexes.

The nucleophilicity parameter, $n_{Pt}$, can be defined as

$$n_{Pt} = \log \left( \frac{k_2(Y)}{k_2(\text{CH}_3\text{OH})} \right) \quad (1.24)$$

The nucleophilicity parameter depends on basicity of ‘$Y$’ towards Pt(II) and oxidation potential [44] of ‘$Y$’. Another feature is that the nucleophilicity of the entering group towards platinum appears to correlate with soft Lewis basicity. The ligands, which are good $\sigma$-donor and $\pi$-acceptor, have high $n_{Pt}$ values.

However, the reaction rates of platinum(II) complexes depend not only on the nucleophilicity of ‘$Y$’, but also on the electrophilicity of the platinum complex. In other
words, different Pt(II) substrates display different abilities to discriminate among nucleophiles. For reactions of nucleophiles ‘Y’ with other Pt(II) complexes in other solvents (S) besides methanol, the relative reactivity of ‘Y’ and ‘S’ is related to $n_{Pt}$ as:

$$\log\left(\frac{k_{2(Y)}}{k_{2(S)}}\right) = S \cdot n_{Pt}$$  \hspace{1cm} (1.25)

The parameter, which characterizes the sensitivity of the rate constant to the nucleophilicity parameter, is called nucleophilic discrimination factor [42]. The large value of ‘S’ indicates that the reaction is more sensitive to changes in nucleophile.

1. (I) 4ae Effect of leaving group

The effect of the leaving group is very difficult to qualify as it is very closely connected with the nature of the incoming nucleophile and the trans ligand. In a dissociative reaction the bond between the leaving group and the metal breaks in the transition state and therefore in these reactions there is a large dependence on the nature of the leaving group. However, for an associative reaction, the effect of the leaving group is dependent on the degree to which bond breaking occurs in the transition state. Each reaction differs in the extent to which bond breaking occurs in the transition. A fairly extensive study of relative effects of leaving group on rates has been made [45] utilizing the reaction (1.26) in aqueous medium.

$$[\text{Pt(dien)}X]^+ + \text{py} \rightarrow [\text{Pt(dien)py}]^{2+} + X^-$$  \hspace{1cm} (1.26)

Here the three coordination positions are rendered inert by using strongly complexing ligand dien (diethylenetriamine) and the entering ligand is pyridine in each case. The order of decreasing rate [45, 46] is $\text{NO}_3^- > \text{H}_2\text{O} > \text{Cl}^- > \text{Br}^- > I^- > \text{N}_3^- > \text{SCN}^- > \text{NO}_2^- > \text{CN}^-$. It has been observed that $\text{H}_2\text{O}$ departs about $10^5$ times faster than $\text{CN}^-$. This indicates that the leaving group has a substantial effect on the rate of the reaction and Pt-X bond breaking make a significant contribution comparable to that of Pt-py bond making. Generally a good attacking group is a poor leaving group with very few exceptions. $\text{OH}^-$ is notable, it is a very poor nucleophile for Pt(II), but is only very slowly replaced.

1. (I) 4af Steric Effect

The first compelling experimental evidence for associative activation in square planar substitution was demonstration of large rate effects on blocking the entering group attack positions above and below the plane. In a square planar complex two ligands cis to
the leaving group and one ligand *trans* to the leaving group are inert ligands. Inert ligands may exert steric hindrance to the incoming nucleophile and may affect the reaction rate. Steric retardation observed in several cases provides good evidence in favour of an associative mechanism, since there is no way to rationalize such retardation on the assumption of a dissociative mechanism. The results of a study of the rates of reaction of *cis*- and *trans*-\([\text{Pt(PEt}_3\text{)}_2\text{(R)Cl}]\) complexes with pyridine in ethanol show the variation of rates in the ratio 1 : 5 : 36 (for \(R = \text{mesityl, o-tolyl and phenyl respectively}\)) in the *trans* complex and the variation of rates in the ratio 1 : 200 : 80,000 (for \(R = \text{mesityl, o-tolyl and phenyl respectively}\)) in the *cis* complex. Thus *cis*-blocking is more effective than *trans*-blocking in associative mechanism.

For the reactions (1.27) carried out in solvents methanol and DMSO, a substantial decrease in \(k_2\) values have been observed [47].

\[
\text{trans-[Pt(ET}_3\text{P)}_2\text{(R)Cl]} + Y \longrightarrow \text{trans-[Pt(ET}_3\text{P)}_2\text{(R)}Y\text{]}^+ + \text{Cl}^- \tag{1.27}
\]

where \(R = \text{phenyl; o-tolyl; 2,6-dimethyl phenyl and Y = CN}^-, \text{SC(NH}_2\text{)}_2\).

A counterbalancing example of the retarding effects of bulky entering group is afforded by the reaction (1.28), in which the use of amines (am) of increasing size progressively diminishes the ‘k’ values [48].

\[
\text{[PtCl}_2\text{(NHEt}_2\text{)(PPr}_3\text{)}]} + \text{am} \rightarrow \text{[PtCl}_2(\text{am})(\text{PPr}_3\text{)}] + \text{NHEt}_2 \tag{1.28}
\]

A very striking example of the large steric effect on the rate of planar substitution is the comparison of the rates of reaction of \([\text{Pt(dien)Cl]}^+\) and \([\text{Pt(ET}_4\text{dien)Cl}^+\) in aqueous solution [6]. In the latter complex, the four ethyl groups on the terminal nitrogens cover above and below the plane and block access to the central metal. It is significant that the hindered complex reacts several orders of magnitude slower than the unhindered one. Apparently both \(k_1\) and \(k_2\) terms are drastically affected, once again strongly suggesting an associative mechanism for the unhindered complex. Indeed, there is no \(k_2\) term in the reactions of entering group with \([\text{Pt(ET}_4\text{dien)Cl}^+\), which indicates that there is no mechanism of ‘a’ type available, and the rate of \(8.5 \times 10^{-6} \text{ sec}^{-1}\) probably represents a dissociative process.

1. (I) 4ag Effect of charge on the complex

A classical test for detecting associative or dissociative pathway is the effect of electronic charge on the reaction rates. Martin and coworkers have provided the most
conclusive evidence for an associative solvent path, with the lack of a large rate effect in 
\( k_1 \) path for reactions of complexes which, carry different net charges in aqueous solution 
\[ 38, 49, 50 \]. Proceeding from \([Pt(NH_3)_2Cl]^+\) to PtCl\(_4^{2-}\), the \( k_1 \) remains roughly constant. This is not consistent with a dissociative path but is reasonable assuming any associative path such as A mechanism.

There are small but significant substrate charge effects on the \( k_2 \) term with certain 
entering groups. In particular, \( k_2(\text{NO}_2^-) \) is larger than \( k_2(\text{Cl}^-) \) with \([Pt(\text{dien})\text{Br}]^+\) as substrate, but \( k_2(\text{Cl}^-) \) is larger than \( k_2(\text{NO}_2^-) \) with \([Pt(\text{dien})\text{OH}_2]^{2+}\) as substrate [46]. The 
inverted order can be explained on the basis of ligand nature. Cl\(^-\) is a \( \sigma \) donor and is a nucleophile, it binds to platinum more effectively with increase in charge; NO\(_2^-\) is a \( \sigma \) donor and an \( \pi \) acceptor. Increase in charge on platinum would inhibit Pt \( \rightarrow \) NO\(_2^-\) \( \pi \)-interaction, hence the rate.

1. (I) 4ah Effect of solvent

The solvent of the reaction medium often influences the energies of the ground 
state and the transition state through solvation. The degree of solvation of the reactants 
and the activated complex has a very pronounced influence on the rate of a reaction. 
Moreover, in planar substitutions, the solvent may act as nucleophile [51] and give rise to 
a parallel path for substitution, called solvent path (Scheme 1.6). Involvement of the 
 solvent molecule in the transition state again supports associative activation. It is to be expected that the contribution made by the solvent path to the overall rate of reaction 
would increase with an increase in the coordinating ability of the solvent. This is in 
accord with the experimental results [52] of the solvent effect on the rate of \( ^{36}\text{Cl}^- \) exchange with \( \text{trans-[Pt(py)}_2\text{Cl}_2\text{]} \). When moderately low concentrations of Cl\(^-\) were used, 
it was observed that the good coordinating solvents provide almost entirely a solvent path 
for exchange (\( k_S \gg k_{\text{Cl}[\text{Cl}^-]} \)). The poorly coordinating solvents contribute little to the overall rate of reaction and the exchange occurs by Cl\(^-\) acting as a nucleophile (\( k_{\text{Cl}[\text{Cl}^-]} \) 
\( \gg k_S \)). For good coordinating solvents the values of \( k_S \) increase in the order ROH < H\(_2\)O 
\( \sim \) CH\(_3\)NO\(_2\) < DMSO. Since the ligand atom sulphur is a better nucleophile than oxygen 
towards Pt(II), the latter forms stable complexes with DMSO and the bonding in the 
transition state [53] is Pt–S, if the role of the solvent were primarily that of solvating the
leaving Cl\(^-\), then H\(_2\)O rather than DMSO would be the more efficient solvent for reaction. Such may be the case if bond breaking were of primary importance. For the \textit{trans-} [Pt(PEt\(_3\))\(_2\)Cl\(_2\)] complex \(k_1(\text{DMSO}) \gg k_1(\text{MeOH})\) [54]. The role of solvent was also illustrated by the substitution of styrene by 1-pentene in \textit{trans-} [Pt(Cl)\(_2\)(PhCH=CH\(_2\))(am)] in chloroform solution (where am = p-substituted anilines and PhCH=CH\(_2\) is styrene) [55]. As ethanol is added to the solvent, the \(k_s\) term becomes important along with a decrease in \(k_2\) values. Presumably solvation of the chloride or the substrate slows down the direct path.

1. (I) 4ai Nature of the Metal Centre

The nature of the reaction centre also affects the rate of substitution of square planar complexes. To ensure that effects are due only to a change in metal centre, only the reactivities of isovalent ions can be compared. Thus, the only d\(^8\) isovalent ions that can be compared are Ni(II), Pd(II), Pt(II) and Co(I), Rh(I) and Ir(I). The general sequence that has been determined is Ni > Pd > Pt in the ratio \(10^7:10^8:10^5:1\). Taube predicts that the rates of reaction decrease with an increase in the stability of the complex.

1. (I) 4aj Effect of pressure

The effect of pressure on the rate of a reaction can be summarized in the simplified expression (1.29):

\[
\ln(k_2/k_1)_T = -\Delta V^\circ(P_2 - P_1)/RT
\]

(1.29)

Here, \(\Delta V^\circ\), the volume of activation, indicates the change in molar volume when the reactants are brought from uncombined state to the transition state, \(k_1\) and \(k_2\) are the rate constants at the pressure \(P_1\) and \(P_2\), usually expressed in megaPascals (Mpa). A concentrated number of studies of pressure effects on the rates of the reactions involving transition metal complexes are now the subject of interest. Instruments are now commercially available and flow, relaxation and NMR techniques have been adopted for use in high pressure kinetics. Fairly high pressures, 200 – 1000 Mpa must be used to obtain the sufficient effect on the rate.
1. (I) 4ak Role of activation parameters

The activation parameters such as enthalpy of activation ($\Delta H^\circ$), entropy of activation ($\Delta S^\circ$) and volume of activation ($\Delta V^\circ$) are related to the transition state of a reaction and play an important role to point out the mechanism. The $\Delta H^\circ$ and $\Delta S^\circ$ are related by Eyring equation:

$$\ln\left(\frac{kh}{k_B T}\right) = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R}$$

(1.30)

Here, ‘k’ is rate constant of the reaction, ‘h’ is Planck constant, ‘$k_B$’ is Boltzman constant, ‘T’ is temperature in Kelvin scale and ‘R’ is universal gas constant. The values of $\Delta H^\circ$ and $\Delta S^\circ$ can be obtained from the slope and intercept of Eyring plot.

Generally for a dissociative activation, $\Delta H^\circ$ has higher value than for an associative activation. Very often low positive value of $\Delta H^\circ$ strongly suggests associative activation. The entropy of activation, $\Delta S^\circ$, is the difference in entropy between the transition state and the ground state of the reactants. In solution where charged particles are involved, solvation effects often dominate the entropy of activation [60]. For associative activation the transition state is more compact than the ground state and a large decrease in entropy is observed i.e. $\Delta S^\circ$ shows large negative value.

$\Delta V^\circ$, volume of activation, is positive for dissociative path and negative for associative path. The values of $\Delta V^\circ$ can be determined from experimental measurements of rates at different pressures. The usual procedure involves a linear plot of $\ln(k_2/k_1)$ against pressure according to equation (1.29) where $P_1$ is atmospheric pressure. From the slope of the plot the value of $(-\Delta V^\circ/RT)$ is obtained and $\Delta V^\circ$ is calculated in cm$^3$mol$^{-1}$ unit. Although $\Delta V^\circ$ is conceptually easy to understand, there is one real problem in its interpretation. This arises because $\Delta V^{\circ}_{\text{obs}}$ can be considered as made up of two parts:

(a) $\Delta V^{\circ}_{\text{intr}}$, the intrinsic volume change when reactants are converted to the activated complex. It arises from changes in bond length; angles etc. and suggest the intimate mechanism.

(b) $\Delta V^{\circ}_{\text{solv}}$, the volume change arising from solvation effects (electrostriction of solvent). Term (b) is unfortunately not easy to assess and this creates problem when it contributes substantially to the overall $\Delta V^{\circ}_{\text{obs}}$. Term (b) is important when the charges of reactants and products are the same and for this reason, exchange reactions have been popular to study for mechanistic information. Activation parameters for substitution
processes of some square planar platinum(II) complexes are illustrated in Table 1.3. Besides the above stated factors, there are other factors also like pH, dielectric constant of the medium, presence of electrolyte etc. which can affect the rate of a substitution reaction.

**Table 1.3:** Kinetic data and activation parameters for substitution reactions of some platinum(II) complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Reagent</th>
<th>$k$</th>
<th>$\Delta H^\circ$</th>
<th>$\Delta S^\circ$</th>
<th>$\Delta V^\circ$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{Pt(H}_2\text{O)}_4]^{2+}$</td>
<td>$\text{H}_2\text{O}$</td>
<td>$3.9 \times 10^{-4}$</td>
<td>90</td>
<td>-9.0</td>
<td>-4.6</td>
<td>34, 35</td>
</tr>
<tr>
<td>$[\text{Pt(dien)Br}]^+$</td>
<td>$I^-$</td>
<td>0.32</td>
<td>46</td>
<td>-104</td>
<td>-</td>
<td>61</td>
</tr>
<tr>
<td>$[\text{Pt(dien)Br}]^+$</td>
<td>$\text{NCS}^-$</td>
<td>0.68</td>
<td>39</td>
<td>-112</td>
<td>-</td>
<td>61</td>
</tr>
<tr>
<td>$[\text{Pt}((\text{dien})\text{Br})]^+$</td>
<td>$\text{SC(NH}_2\text{)}_2$</td>
<td>1.30</td>
<td>35</td>
<td>-121</td>
<td>-</td>
<td>61</td>
</tr>
<tr>
<td>$\text{trans-[Pt(py)}_2(\text{Cl})(\text{NO}_2)]^p_y$</td>
<td>$\text{py}$</td>
<td>$7.35 \times 10^{-3}$</td>
<td>49.3</td>
<td>-94</td>
<td>-8.8</td>
<td>62</td>
</tr>
<tr>
<td>$\text{cis-[Pt(py)}_2(\text{Cl})(\text{NO}_2)]$</td>
<td>$\text{py}$</td>
<td>$0.28 \times 10^{-3}$</td>
<td>46.8</td>
<td>-129</td>
<td>-5.5</td>
<td>62</td>
</tr>
<tr>
<td>$\text{trans-[Pt(PEt}_3\text{)}_2\text{Cl}_2]$</td>
<td>$\text{py}$</td>
<td>$0.53 \times 10^{-3}$</td>
<td>53.9</td>
<td>-100</td>
<td>-13.6</td>
<td>62</td>
</tr>
<tr>
<td>$[\text{Pt(dien)(H}_2\text{O})]^{2+}$</td>
<td>$\text{Br}^-$</td>
<td>2.26</td>
<td>47.5</td>
<td>-92</td>
<td>-</td>
<td>63</td>
</tr>
<tr>
<td>$[\text{PtCl}_4]^{2-}$</td>
<td>$\text{DMSO}$</td>
<td>$2.8 \times 10^{-3}$</td>
<td>63.6</td>
<td>-96</td>
<td>-</td>
<td>64</td>
</tr>
<tr>
<td>$[\text{PtCl}_4]^{2-}$</td>
<td>$\text{thiooxan}$</td>
<td>$4.4 \times 10^{-3}$</td>
<td>46.4</td>
<td>-142</td>
<td>-</td>
<td>64</td>
</tr>
<tr>
<td>$[\text{PtCl}_4]^{2-}$</td>
<td>$\text{SCN}^-$</td>
<td>$3.5 \times 10^{-3}$</td>
<td>48.6</td>
<td>-130</td>
<td>-</td>
<td>65</td>
</tr>
</tbody>
</table>

$a$ in s$^{-1}$.

1. **(I) 5 Dissociative Mechanism**

As it has already been discussed, a dissociative mechanism is not the favoured pathway for substitution of square planar complexes; however, it can be encouraged by following one or more of the following methods: promote bond weakening, stabilize the intermediate of lower coordination number or prevent bond formation. In theory this can be done by using either electronic or steric effects.

The presence of $k_1$ term in the rate equation of square planar substitution $k_{obs} = k_1 + k_2[Y]$, where the value of $k_1$ is independent of the ligand nature does not definitely prove the participation of the solvent path, because a dissociative path involving M–X bond breaking may also lead to the same result according to the Scheme 1.8.
The rate law for the mechanism is:

\[
d[ML_3Y]/dt = k_2 [ML_3][Y]
\]

Applying steady state approximation for [ML_3]

\[
d[ML_3]/dt = 0 = k_1 [ML_3X] - k_1 [ML_3][X] - k_2 [ML_3][Y]
\]

[ML_3] = \frac{k_1 [ML_3X]}{(k_1[X] + k_2[Y])}

Rate = \frac{(k_2 k_1 [ML_3X][Y])}{(k_1[X] + k_2[Y])}

When \( k_1[X] \ll k_2[Y] \)

\[
\text{Rate, } k = \frac{k_1 [ML_3X]}{k_2[Y]}
\]

\[ k_{obs} = k_1 \] (1.31)

In case of Pt(II) complexes such a dissociative path operates only when the substrate is so much sterically hindered that the incoming ligand finds no way to approach the metal. Such steric blocking has almost been achieved with the complex \([Pt(Et_4dien)Cl]^+\). The four terminal ethyl groups are above and below the plane and render virtually impossible any access to the central metal.

For the reaction (1.32), the value of \( k_{obs} \) is \( 8.5 \times 10^{-6} \) sec\(^{-1}\) and the rate at 80°C is zero order in reagent concentration [66].

\[
[Pt(Et_4dien)Cl]^+ + py \rightarrow [Pt(Et_4dien)py]^{2+} + Cl^- \] (1.32)

For the reaction Scheme 1.9

\[
\text{Cis-[Pt(C_6H_5)_2(Me_2SO)_2] + L-L} \rightarrow \text{Cis-[Pt(C_6H_5)_2(Me_2SO) (L-L)] + Me_2SO}
\]

\[
\text{fast}
\]

\[
\text{Cis-[Pt(C_6H_5)_2(L-L)] + Me_2SO}
\]

\[ \text{Scheme 1.9} \]

Where L-L (a bidentate ligand) and Me_2SO are used in excess, the pseudo first order rate constant ‘k’ for the loss of the Pt(II) reactant is
\[ k = \frac{a[L-L]}{b[L-L] + [\text{Me}_2\text{SO}]} + c[L-L] \quad (1.33) \]

\( a = k_1k_2/k_{-1}, \quad b = k_2/k_{-1} \) and \( c = k_2 \). Markedly different values of ‘b’ for different entering ligands \((L-L)\) support a D mechanism [20]. The D mechanism might be favored in very poor nucleophilic solvents such as \( \text{C}_6\text{H}_6 \) or \( \text{CHCl}_3 \), but its differentiation from the solvolytic path is still very difficult [9, 67, 68]. The \( \Delta V^\circ \) value of \((5.5 \pm 0.8) \text{ cm}^3\text{mol}^{-1}\) for the exchange of \( \text{Me}_2\text{SO} \) with \( \text{cis-}[\text{Pt}(\text{C}_6\text{H}_5)_2(\text{Me}_2\text{SO})_2] \) also strongly supports a D mechanism which appears favored in complexes containing Pt-\( \text{C} \) bond [69].

However, the most convincing evidence for the operation of D mechanism rather than an interchange substitution process comes from \( \text{cis} = \text{trans} \) isomerization studies [18]. For the reaction \((1.34)\):

\[
\begin{align*}
\text{cis-}[\text{PtL}_2\text{RCI}] & \rightleftharpoons \text{trans-}[\text{PtL}_2\text{RCI}] \\
\text{where L is PEt}_3 \text{ and R is aryl ligand, the following mechanism was suggested for the observed kinetics}
\end{align*}
\]

\[
\begin{align*}
\text{cis-}[\text{PtL}_2\text{RCI}] & \xrightarrow{k_d/k_t} \text{cis-}[\text{PtL}_2\text{R}] + \text{Cl}^- \\
\text{cis-}[\text{PtL}_2\text{R}] & \xrightarrow{k_t/k_a} \text{trans-}[\text{PtL}_2\text{R}] \\
\text{trans-}[\text{PtL}_2\text{R}] + \text{Cl}^- & \xrightarrow{k_a/k_t} \text{trans-}[\text{PtL}_2\text{RCI}] 
\end{align*}
\]

The isomerisation equilibrium lies well to the right and both \( k_{-t} \) and \( k_{-a} \) are negligible. The observed rate constant is, \( k_{\text{obs}}^{\text{Isom}} = [(k_t/k_d)(k_d[\text{Cl}^-] + k_t)] \)

\[
\text{when } k_d[\text{Cl}^-] \ll k_t, \quad k_{\text{obs}}^{\text{Isom}} = k_d \quad (1.39)
\]

For the three coordinated isomers \( \text{cis-[Pt(Et}_3\text{P)}_2\text{R}]^+ \) and \( \text{trans-[Pt(Et}_3\text{P)}_2\text{R}]^+ \) a T-shaped structure has been proposed [70, 71]. The kinetic results of the reactions of \( \text{cis-[PtMe}_2(\text{DMSO})_2] \) with bromazepam and bipyridyl in DMSO and in \( \text{CH}_2\text{Cl}_2 \) have been interpreted in terms of a dissociative mechanism [72].

Photochemical isomerisation [73] of the complex \( \text{cis-[Pt(PPh}_3)_2\text{Cl}_2] \) proceeds through a dissociative path according to the Scheme 1.10.
Scheme 1.10

The aqua ligand displacement reaction between cis-[Pt(H₂O)₂(NH₂CHMe₂)₂]²⁺ and 5'-guanosine monophosphoric acid also shows a positive ΔS° value for the second step of substitution indicating a dissociative mechanism for this step [74].

1. (I) 6 Assessment of the mechanism for square planar substitutions

It is now established beyond doubt that the intimate mechanism of square planar substitution is a type i.e. the energy of the transition state is profoundly affected by the nature of the entering group. The theoretical considerations lead to the conclusion that planar d⁸ substitutions are ideal cases for an associative mechanism involving a five coordinated intermediate or transition state. Isolation of such intermediate depends on the presence of deep potential wells along the reaction profile. The presence of such minima depends on a number of factors, which are difficult to understand and cannot be easily predicted. For example, there is good evidence that all the reactions of tertiary phosphines with bis-β-diketonato complexes of Pt(II) and Pd(II) proceed via five coordinated species. Though some can be isolated, others can only be detected spectroscopically and many cannot be observed at all [75-77].

A necessary consequence of assigning these five coordinated species as intermediate or transition state is that the five coordinated d⁸ molecules show a tendency to lose a ligand. For example, NMR spectroscopic measurements show that five coordinated [PdBr₂(PMe₃)₃] and [PdBr(PMe₃)₄]⁴⁺ readily lose Br⁻ and PMe₁ respectively
in CD$_2$Cl$_2$ solution [32]. Several examples of four and five coordination equilibrium of the type (1.40) have been detected by variable temperature NMR spectroscopy [33].

\[
[\text{PtX}_2(\text{PR}_3)_2] + \text{PR}_3 \rightleftharpoons [\text{PtX}_2(\text{PR}_3)_3]
\]  
(1.40)

For the five coordinated species there are two possibilities, depending on whether the detailed mechanism is \( A \) or \( I_a \). In the \( A \) process, there is a minimum in the plot of free energy versus reaction coordinate, corresponding to a symmetrical (D$_{3h}$) trigonal-bipyramidal structure. In the \( I_a \) process, the symmetric trigonal bipyramid is the highest energy structure and is the transition state. The validity of the \( A \) mechanism is certainly strongly suggested by the impressive correlation of a wide variety of substituent effects based on a process that proceed through an approximately trigonal bipyramidal transition state and a trigonal bipyramidal intermediate. Intimate analysis of the five coordinate transition state shows that the three ligands, that occupy the trigonal plane in the trigonal bipyramidal, may take advantage of certain \( \sigma \)- and \( \pi \)-bonding possibilities which are substantially changed from the ground state square planar complex. On the other hand, the bonding situation of the apical ligands is not appreciably different from the ground-state complex. Thus, the assumption of an approximately trigonal bipyramidal geometry for the transition state correctly predicts relatively large substituent effects for the ligands in the trigonal plane (the trans, entering, and leaving groups) and relatively small effects for the apical ligands (the cis groups).

1. **Summary of Pt(II) substitution reactions**

   The activation process of square planar substitution reactions can be divided into two categories – (i) associative (A) and (ii) dissociative (D). The major process is associative in nature. The assumption of an A mechanism via a trigonal bipyramidal intermediate is able to account almost all the evidences available so far. The main evidence for the associative trigonal bipyramidal mechanism for the \( k_2 \) path is as follows:

1. a unified interpretation of substituent effects based on the model of an \( A \) process;
2. the decrease in observed rate on blocking the attack positions. It is found that \textit{cis} blocking is more effective than \textit{trans} blocking;
3. the large entering group effect which closely parallels the trans effect order of the ligands;
4. the observation that no [Pt(dien)OH]+ is formed during the reaction of [Pt(dien)Br]+ with Y in the presence of OH−, showing that there can be no intervention of the solvent along the k2 path.

The main evidence for the associative trigonal bipyramidal mechanism involving solvent as reagent in the k1 term is as follows:

1. the large decrease in the k1 rate on blocking the attack position in several Pt(II) complexes;
2. the observation that the k1 rate is relatively insensitive to changes in the net charge on the complexes;
3. solvent effect experiments that show good coordinating solvent enhance the k1 term, e.g., k1(DMSO) > k1(H2O) in reactions with PtA2Cl2 complexes.

The dissociative path operates when the substrate is so much sterically hindered that the incoming ligand finds no way to approach the metal centre.
References

References are in the following format: Author, *Journal*, Volume, Page number (Year).

Section II
Some recent studies on ligand substitution reactions of square planar platinum(II) complexes

Studies on the substitution reactions of square-planar complexes of Pt(II) in particular have received much attention from various investigators over the last few decades. The interest in this field continues uninterruptedly as demonstrated by the large number of papers appearing annually. This interest mainly focuses on the ability to use steric and electronic effects to tune the solubility, acidity and reactivity of such complexes for their application as anti-tumour drugs.

It is recently reported that mono-functional Pt(II) complexes with tridentate ligands, e.g. terpyridine, show remarkable trends in reaction rates, pKa values and nucleophilic discrimination depending on the π-acceptor properties of the spectator ligands [1]. Since a bifunctional addition to DNA seems to be critical for its cytotoxic activity, therefore Summa et al. selected a series of Pt(II) complexes with either a different combination of amines or both, amines and pyridines in a bidentate fashion [2]. [Pt(ethylenediamine)(H₂O)₂]²⁺ (Pt(en)) was used as a model complex for cisplatin, only circumventing facile substitution of amines by using a diamine chelate. The complex [Pt(diaminocyclohexane)(H₂O)₂]²⁺ (Pt(dach)) can be considered as the hydrolysis product of oxaliplatin ((trans-1,2-diaminocyclohexane)oxalatoplatinum(II)), a third generation platinum drug. It was found to be active in cisplatin resistant tumors [3]. To continue the investigations of Hofmann et al. in terms of the effect of π-acceptors on the thermodynamic and kinetic properties of Pt(II) complexes, Summa et al. combined half of the Pt(en) complex with one π-acceptor ligand, resulting in [Pt(aminomethylpyridine)(H₂O)₂]²⁺ (Pt(amp)). Regarding the nature and electronic properties of the amines (NH₂R, pyridine), the Pt(amp) complex can be considered as a model for cis-[PtCl₂(NH₃)(2-picoline)] (AMD473), although the methyl group of the picoline ligand in AMD473 will have an additional steric influence on the reaction rate, most likely a deceleration. AMD473 entered clinical trials Phase I and II and shows a profile of chemical and biological activity that differs significantly from that of cisplatin [4]. Finally, in [Pt(N,N’-bipyridine)(H₂O)₂]²⁺ (Pt(bpy)) two pyridine rings were
introduced to further study the influence of π-acceptors on the electronic properties of the Pt(II) center. Summa et al. investigated the reactions of the complexes Pt(dach), Pt(en), Pt(amp), and Pt(bpy) with tu, L-Met, and 5′GMP using UV-Vis spectrophotometric and stopped-flow techniques to study the influence of electronic effects on reactions of potential biological importance. The reactivity order for the Pt(II) aqua complexes (viz., Pt(dach) ≈ Pt(en) < Pt(amp) << Pt(bpy)) confirms what was already expected on the basis of the pKa values (viz., the obvious influence of the diamine spectator ligands on the reactivity of the Pt(II) center) [2]. Addition of π-acceptors to the complex results in an increased reactivity of the Pt(II) center, due to their electron-withdrawing properties, which results in increasing reaction rates for nucleophilic substitution reactions. Especially when two pyridine rings are adjacent to each other as in Pt(bpy), the rates increase significantly (compared to Pt-(dach)/Pt(en)) by a factor of about 30-150, compared to a factor of 3-7 in the case of only one π-acceptor (Pt(amp)). This can be attributed to the stronger trans effect of π-acceptors such as pyridine rings compared to the weak trans effect of amines of the type NH₂R, NHR₂. These findings are also reflected in the pKa values of the coordinated water molecules, since they decrease with an increasing number of pyridine rings. Thus, hydroxo species can be stabilized more efficiently by pyridine-containing chelates than by RNH₂ chelates. Therefore, the pH can control the reactivity of the complex over the fraction of the aqua complex available in solution, which is important, considering that the hydrolysis product is the active species in the mechanism of action of Pt(II) antitumor drugs. From a comparison of the reactivity of tu, L-Met, and 5′GMP in the reaction with the different Pt(II) complexes, it can be concluded that with increasing electrophilicity the reactivity is increased by an enhanced nucleophilic discrimination of the complex. While tu is clearly the strongest nucleophile, the N-donor nucleophile 5′GMP exhibits an affinity for Pt(II) complexes which is as good as the S-donor L-Met under these conditions. Although the HSAB theory favors a stronger interaction of Pt ions with S-donor ligands, it seems that the conditions under which the reactions proceed are very important for the kinetic competition of N- and S-donor ligands [5]. At pH 2.0 - 2.2, 5′GMP will react as a monoanionic species, while L-Met (pKa = 2.13, 2.28) is up to 42% cationic and 58% neutral. In this case 5′GMP reacts faster than L-Met [2].
Coordination compounds of platinum(II) with tridentate ligands such as bis(2-pyridylmethyl)amine (bpma) provides very useful substrates for studies on ligand substitution reactions of square-planar complexes [6]. Jaganyi et al. investigated the reactions of the complex [Pt(bpma)(OH$_2$)]$^{2+}$ with thiourea (TU), 1,3-dimethyl-2-thiourea (DMTU), 1,1,3,3-tetramethyl-2-thiourea (TMTU), Cl$^-$, Br$^-$, I$^-$ and SCN$^-$ [6]. Based on the second order rate constants, it was concluded that the reactivity of the nucleophiles towards the complex follows the order: I$^-$ > DMTU > TU > SCN$^-$ > TMTU > Br$^-$ > Cl$^-$. This reactivity order depends on the polarizability of the incoming ligand and the softness or hardness of the metal; iodine being the most polarized halide reacts much faster than the other nucleophiles. The entropy values for [Pt(bpma)(OH$_2$)]$^{2+}$ are negative which confirms that the mechanism is associative as expected.

The kinetics of the substitution reactions of four different mononuclear Pt(II) complexes, viz. cis-[Pt(NH$_3$)$_2$Cl$_2$], [Pt(SMC)Cl$_2$]$^-$ (in the deprotonated form), [Pt(en)Cl$_2$], and [Pt(dach)Cl$_2$], were investigated under physiological conditions at 310 K and pH = 7.2 in Hepes buffer with selected biologically important ligands, viz. guanosine-5'-monophosphate (5'GMP), L-histidine and 1,2,4-triazole by Bogojeski et al. (where SMC = S-methyl-L-cysteine, en = ethylenediamine and dach = 1,2-diaminocyclohexane) [7]. Two consecutive reaction steps, both depend on the nucleophile concentration, were observed in all cases. The second-order rate constants for both reaction steps indicate a decrease in the order [Pt(SMC)Cl$_2$]$^-$ > cis-[Pt(NH$_3$)$_2$Cl$_2$] > [Pt(en)Cl$_2$] > [Pt(dach)Cl$_2$]. Those calculations collectively supported the experimentally observed substitution of thioethers bound to Pt(II) complexes by N7(5’GMP). The studied Pt(II) complexes have a high affinity for the studied N-bonding nucleophiles of which 1,2,4-triazole is a better nucleophile than 5’GMP and l-histidine. DFT calculations (B3LYP/LANL2DZp) showed that the Pt–N7(Guo) adduct is more stable than the Pt–S(thioether) adduct for the studied complexes cis-[Pt(NH$_3$)$_2$Cl$_2$], [Pt(SMC)Cl$_2$]$^-$, and [Pt(en)Cl$_2$]. For the first step in the gas phase Pt–N7(Gua) is more stable than Pt–S(thioether) by ca. 31–33 kcal/mol, and for the second step by 32–34 kcal/mol. The calculations collectively supported the experimentally observed substitution of thioethers bound to Pt(II) complexes by N7(5’GMP).
The substitution behaviour of the [Pt(2-methylthiomethylpyridine)(OH$_2$)$_2$]$^{2+}$ complex have been studied for the sulphur-containing nucleophiles thiourea (tu), N,N'-dimethylthiourea (dmtu) and N,N,N',N'-tetramethylthiourea (tmtu), which differ significantly in their steric hindrance during nucleophilic attack [8]. The kinetic measurements have been studied spectrophotometrically by following the change in absorbance at suitable wavelengths as a function of time. Different pH values were chosen to investigate the different generated diaqua, aqua-hydroxo and dihydroxo species. A reaction scheme was postulated to explain the different observations at different pH values. In general the first reaction step takes place trans to the sulphur donor atom, because sulphur has a strong trans-labilisation effect on the water molecule. At pH 2, the predominant species is the diaqua Pt(mtp) complex, which shows a clear first substitution step with no intercept for all nucleophiles. The second observed reaction includes two steps, namely the displacement of the second water ligand and dechelation of the pyridine ring. Although these two steps could not be separated kinetically, the final product has been verified using mass spectrometric measurements. The values of the rate constants at pH 2 for the first substitution step $k_1$ decrease in the order tu (634 mol dm$^{-3}$ s$^{-1}$) > dmtu (507 mol dm$^{-3}$ s$^{-1}$) > tmtu (165 mol dm$^{-3}$ s$^{-1}$). Ligand tu and dmtu show almost similar reactivity whereas tmtu, the sterically most hindered nucleophile, shows by far the lowest reactivity. In addition, temperature and pressure dependent studies were performed. The acceleration of the reactions by pressure and the significantly negative V* values are typical for associative substitution reactions, which is also supported by the negative S* values found for the reactions with all nucleophiles.

The kinetics and mechanism of substitution reactions of novel monofunctional [Pt(tpdm)Cl]$^+$ complexe (where tpdm = tripyridinedimethane) and their aqua analogues with thiourea (tu), L-methionine (L-met), glutathione (GSH), and guanosine-5'-monophosphate (5'GMP) have been studied in 0.1 M NaClO$_4$ at pH = 2.5 (in the presence of 10 mM NaCl for reactions of the chloride complexes) [9]. The reactivity of the investigated nucleophiles follows the order tu > L-met > GSH > 5'GMP. The reported rate constants showed the higher reactivity of the aqua complex than the corresponding chlorido complex. The negative values reported for the activation entropy as well as the activation volume confirmed an associative substitution mode. In addition, the molecular
and crystal structure of [Pt(tpdm)Cl]Cl was determined by X-ray crystallography. Due to the tetrahedral arrangement around the methylene groups the pyridine ligands are forced to be out of plane with the metal center, which have a significant effect on their π-back-bonding ability and as a result the lability of the complexes. Whereas π-back-bonding properties of the terpy chelate can account for the acceleration of the nucleophilic substitution process as compared to the tpdm chelate where introduction of two methylene groups prevents such an effective π-back-bonding.

A series of novel dinuclear Pt(II) complexes with bidentate nitrogen donor ligands and two vacant coordination sites were synthesized by Hochreuther et al. [10]. Thereby, the effect of increasing the length of the aliphatic chain on the bridged complexes was studied. Determination of the pKa values showed four acid dissociation steps. Because of the overall charge of 4+ on the complexes, the water ligands are more acidic compared to the mononuclear reference complex Pt(amp). Furthermore, a deprotonation pattern was established where the first and second steps occur trans to the pyridine unit. The difference between the pKa₁ and pKa₂ values, as well as between the pKa₃ and pKa₄ values, becomes smaller as the distance between the Pt(II) centers increases. This is assigned to an electrostatic interaction between the two Pt(II) centers, which becomes weaker on elongation of the chain. Substitution kinetics was performed with thiourea. Because of the neutral character of the nucleophile, the overall charge does not change during the substitution reactions and two successive steps could be observed. It was found that the first reaction step, k₁, is independent of the chain length, because the driving force in this case is the strong π-acceptor ability of the pyridine ligand. In contrast, the second substitution step, k₂, depends on the chain length, because elongation of the chain leads to an electron donating effect, which results in a less electrophilic Pt(II) center that slows down the reaction. This study has shown that it is necessary to balance the different donor abilities of the coordinated ligands to determine which effect predominates. It is found that the π-acceptor pyridine overruled the σ-donor NHR₂ contribution and caused the entering nucleophile to be stabilized in the position trans to the pyridine unit. The acceleration of the reactions by pressure and the significantly negative V# values are typical for an associative substitution mechanism, which is also
supported by the negative $\Delta S^\circ$ values calculated from the temperature-dependent studies of the reaction.

The substitution behaviour of a series of novel dinuclear platinum(II) complexes containing a mixed nitrogen and sulfur donor bidentate chelate systems were studied as a function of entering thiourea concentration, pressure and temperature using stopped-flow techniques and UV–Vis spectroscopy. The two platinum centers are connected by an aliphatic chain of variable length by which the effect of elongating the methylene chain was studied. Furthermore, this study was interesting because of the influence of combining a strong $\sigma$-donor and $\pi$-acceptor within one complex system. With elongation of the chain, more electron density is pushed onto the metal center, which leads to slower substitution reactions.

The specific hydrolysis of histidine-containing peptides promoted by cis-[Pt(en)(H$_2$O)$_2$]$^{2+}$ has been investigated by electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance spectrometry (NMR) [12]. MS and $^1$H NMR studies confirmed that the histidine containing tripeptides can only be hydrolyzed specifically at the first downstream peptide bond from histidine in the presence of cis-[Pt(en)(H$_2$O)$_2$]$^{2+}$, a rare example where Pt(II) complex act as peptidase. ESI-MS tracking disclosed that the formation of Pt-anchored peptides is the key step for the hydrolysis, and the exhaustion of the Pt(II)-anchored intermediate results in the end of hydrolysis. Moreover, more Pt(II) complex anchored on to peptide leads to more hydrolysis and higher hydrolysis rate. In addition, the formation of Pt-anchored intermediates is quicker in the case of AcGHL than in AcGHG. On the other hand, $^1$H NMR study indicated that Pt(II)-anchoring to the peptide via coordinating to the imidazole N atom and the peptides are hydrolyzed in apparent first-order kinetics. The hydrolysis rate of AcGHG is larger than that of AcGHL, although the Pt-anchored AcGHL intermediates are formed more quickly.

The biological activity of four cisplatin-like Pt–phosphane complexes, namely, cis-[PtCl$_2$(L)$_2$], L = PPh$_3$, P(Ph)$_2$(p-C$_6$H$_4$-COOH), P(Ph)$_2$(CH$_2$CH$_2$-COOH) and its succinimidyl ester derivative, has been tested by Ravera et al. on monolayer cultures of three tumour cell lines (namely, A2780 human ovarian carcinoma and its cisplatin-resistant form A2780Cp8, and human colon adenocarcinoma HCT116) [13]. These
complexes can undergo intramolecular rearrangements by virtue of their functionalized phosphanes, thereby existing as fully opened (O) or fully closed (C) forms. These results showed that only the opened forms exhibit moderate activity, which, although inferior to the activity exhibited by the archetype metallo-drug cisplatin, is substantially retained in the A2780Cp8 cell line, yielding very low resistance factors. The trend is also maintained on the less cisplatin-sensitive HCT116 line. When the complexes assume the bis-chelated (C) form, the antiproliferative activity is deeply reduced. Two Pt-amine congeners, containing β-alanine and 3-methoxypropylamine, with C and O structures, respectively, were synthesized and their antiproliferative propensity was evaluated for comparison purposes, and a similar scenario was observed.

Carboplatin, an analogue of ‘‘classical’’ cisdiamminedichloridoplatinum(II) (cisplatin), is a widely used second-generation platinum anticancer drug. Cytotoxicity of cisplatin and carboplatin is mediated by platinum–DNA adducts. Markedly higher concentrations of carboplatin are required, and the rate of adduct formation is considerably slower. The reduced toxic effects in tumor cells and a more acceptable side-effect profile are attributable to the lower reactivity of carboplatin with nucleophiles, since the cyclobutanedicarboxylate ligand is a poorer leaving group than the chlorides in cisplatin. Recently, platinum complexes were shown to be particularly attractive as potential photochemotherapeutic anticancer agents. Selective photoactivation of platinum complexes by irradiation of cancer cells may avoid enhancement of toxic side-effects, but may increase toxicity selectively in cancer cells and extend the application of photoactivatable platinum complexes to resistant cells and to a wider range of cancer types. Therefore, it was of interest to examine whether carboplatin can be affected by irradiation with light to the extent that its DNA binding and cytotoxic properties are altered. Mlouskova et al. have found that carboplatin is converted to species capable of enhanced DNA binding by UV irradiation and consequently its toxicity in cancer cells is markedly enhanced [14]. Recent advances in laser and fiber-optic technologies make it possible to irradiate also internal organs with light of highly defined intensity and wavelength. Thus, carboplatin is a candidate for use in photoactivated cancer chemotherapy.
Picoplatin is a sterically hindered mononuclear platinum drug undergoing clinical trials. The 2-methylpyridine ring provides steric hindrance to the drug, preventing attack from biological nucleophiles. BBR3464 is a trinuclear platinum drug which was recently in Phase II clinical trials, and is highly cytotoxic both in vitro and in vivo; it derives this activity through the flexible adducts it forms with DNA. Brown et al. have sought to combine the properties of both drugs to synthesise a family of sterically hindered, dinuclear platinum complexes as potential anticancer agents [15]. The bis-pyridyl-based ligands were synthesized through a peptide coupling reaction using diaminoalkanes of differing lengths (n = 2, 4 or 8) and 4-carboxypyridine or 2-methyl-4-carboxypyridine. The resultant dinuclear platinum complexes were synthesized by reacting two equivalents of transplatin or mono-aquated transplatin to each ligand, followed by purification after precipitation with acetone. The unprotected complexes react faster with 5′-guanosine monophosphate (drug to nucleotide ratio 1: 2; t_{1/2} = 2 h), glutathione (1: 10, t_{1/2} = 55 min) and human serum albumin (HSA) (1: 1, t_{1/2} = 24 h) compared to their hindered, protected equivalents (5′-guanosine monophosphate, t_{1/2} = 3.5 h; glutathione = 1.7 h; HSA, t_{1/2} = 110 h). The complexes were tested for in vitro cytotoxicity in the A2780 and A2780/cp70 ovarian cancer cell line. The unprotected platinum complexes were more cytotoxic than their protected derivatives, but none of the complexes were able to overcome resistance. The results provided important proof-of-concept for the development of a larger family of sterically hindered multinuclear-based platinum complexes.

The binding mechanism of second (carboplatin) and third generation (oxaliplatin and nedaplatin) anticancer drugs with guanine (G) and adenine (A) DNA bases, under both neutral and acidic conditions, has been investigated using density functional theory (DFT) combined with the conductor-like dielectric continuum model (CPCM) approach [16]. The work make a direct comparison between the rates of formation of the mono-functional adducts of these compounds. The guanine as a target for platination process was confirmed to be preferred over adenine for all the investigated compounds. The dominating preference for G purine seems to be a hydrogen-bond-controlled process, confirming that H-bonds are important in imposing both structural and kinetic control on the purine platination processes. Moreover, the lower energy of the N7 lone-pair MO on guanine ring compared with that observed for adenine permits a more favorable donor-
acceptor interaction with the Pt(II) fragment. In both environments, carboplatin showed the lowest activation barrier for the G-platination process and seems to be a direct consequence of the network of hydrogen bonds that takes place in the transition state geometry. Also in this case, the electronic behavior of the donor orbital located on the nucleophilic N7 atoms on the guanine ring contributes to the stabilization of the transition state.

Substitution reactions of the complexes [Pt(bpma)H₂O]²⁺ and [PtCl(bpma)]⁺, where bpma is bis-(2-pyridylmethyl)amine, with the nitrogen-donor ligands 1,2,4-triazole, pyrazole and pyridazine were studied in aqueous 0.1 M NaClO₄ using variable-temperature UV–Vis spectrophotometry by Bogojeski et al. [17]. The reactions of the aqua complex were studied at pH 2.5, and those of the chloro complex were studied in the presence of 10 mM NaCl to prevent their hydrolysis. The values obtained for the second-order rate constants indicate that the complexes with bpma are more reactive than those with diethylenetriamine. In both cases, the aqua complexes are more reactive than the corresponding chloro complexes. The reactivity of the incoming ligands follows the order: 1,2,4-triazole > pyridazine > pyrazole. Activation parameters were determined for all the reactions, and the negative entropies of activation (∆S*) support an associative mechanism.

The substitution reaction of the Pt(IV) complex [PtCl₄(bipy)] with guanosine-5′-monophosphate was studied by UV–Vis spectrophotometry [18]. This reaction was investigated under pseudo-first-order conditions at 37 °C in 25 mM Hepes buffer (pH = 7.2) in the presence of 10 mM NaCl to prevent the hydrolysis of the complex. The substitution of chlorides in [{trans-Pt(NH₃)₂Cl}₂(-1,2-bis(4-pyridyl)ethane)](ClO₄)₂ complex by guanosine-5′-monophosphate was followed by ¹H NMR spectroscopy under second-order conditions. Very similar values for the rate constants of both substitution steps were obtained. The Pt(IV) complexes, [PtCl₄(bipy)] and [PtCl₄(dach)], as well as dinuclael Pt(II) [{trans-Pt(NH₃)₂Cl}₂(-pyrazine)](ClO₄)₂, [{trans-Pt(NH₃)₂Cl}₂(-4,4′-bipyridyl)](ClO₄)₂ DMF and [{trans-Pt(NH₃)₂Cl}₂(-1,2-bis(4-pyridyl)ethane)](ClO₄)₂ complexes, displayed potent cytotoxic activity against human ovarian carcinoma cell line TOV21G and lower activity toward human colon carcinoma HCT116 cell line at the
same concentrations. Those data indicated that those platinum complexes could be explored further, as potential therapeutic agents for ovarian cancer.

The novel dinuclear Pt(II) complexes \([\text{trans-Pt(NH}_3_2\text{Cl}_2(\text{-pyrazine})}\text{(ClO}_4\text{)}_2] (\text{Pt1}), \quad [\text{trans-Pt(NH}_3_2\text{Cl}_2(\text{-4,4'-bipyridyl})}\text{(ClO}_4\text{)}_2\cdot\text{DMF} (\text{Pt2}), \quad \text{and} \quad [\text{trans-Pt(NH}_3_2\text{Cl}_2(\text{-1,2-bis(4-pyridyl)ethane})}\text{(ClO}_4\text{)}_2] (\text{Pt3}), \) were synthesized by Soldatovic et al. Acid–base titrations, and temperature and concentration dependent kinetic measurements of the reactions with biologically relevant ligands such as thiourea, glutathione, and guanosine-5'-monophosphate, were studied at pH 2.5 and 7.2. The reactions were followed under pseudo-first-order conditions by stopped-flow and UV-Vis spectrophotometry. \(^1\text{H} \) NMR spectroscopy was used to follow the substitution of chloride in the complex \([\text{trans-Pt(NH}_3_2\text{Cl}_2(\text{-4,4'-bipyridyl})}\text{(ClO}_4\text{)}_2\cdot\text{DMF} \) by guanosine-5'-monophosphate under second-order conditions. The results indicated that the bridging ligand has an influence on the reactivity of the complexes towards nucleophiles. The order of reactivity of the investigated complexes is \(\text{Pt1} > \text{Pt2} > \text{Pt3} \).

The complex \([\text{Pt}_2(\text{N}^1,\text{N}^{10}\text{-bis(2-pyridylmethyl)-1,10-decanediamine})(\text{OH}_2)_4]^{4+} \) consists of a bidentate N,N-donor chelating ligand system in which the two platinum centers are connected by an aliphatic chain of 10 methylene groups is of further special interest, since only little is known about the substitution behavior of such dinuclear platinum complexes that contain a bidentate coordination sphere. The substitution behavior of such dinuclear platinum complexes was investigated using different biologically relevant nucleophiles, such as thiourea (tu), L-methionine (L-Met), glutathione (GSH), and guanine-5'-monophosphate (5'GMP), at two different pH values (2 and 7.4) [20]. The substitution of coordinated water by these nucleophiles was studied under pseudo-first-order conditions as a function of nucleophile concentration, temperature, and pressure, using stopped-flow techniques and UV–Vis spectroscopy. The reactivity of the complex with the selected nucleophiles was found to be \(\text{tu} \gg 5'\text{GMP} > \text{L-Met} > \text{GSH} \) at pH 2 and \(\text{GSH} > \text{tu} > \text{L-Met} \) at pH 7.4. The results for the dinuclear complex were compared to those for the corresponding mononuclear reference complex \([\text{Pt(aminomethylpyridine)}(\text{OH}_2)_2]^{2+}, \) Pt(amp), by which the effect of the addition of an aliphatic chain, an increase in the overall charge, and a shift in the pKa values of the
coordinated water ligands could be investigated [2]. The reactivity order for Pt(amp) was found to be tu > GSH > L-Met at pH 7.4.
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Section III
Platinum–amine chemistry in reference to anticancer activity

Cisplatin was first synthesized in 1844 by Peyrone, in Turin, but its biological activity was accidentally discovered in 1965 by Rosenberg [1-3]. During the investigation of the influence of weak alternating currents on the growth of *Escherichia coli* bacteria, Rosenberg used ostensibly inert platinum electrodes. The result of these experiments was an inhibition of cell reproduction without simultaneous inhibition of bacterial growth, which eventually led to the formation of long, filamentous cells. After extensive research, they realized that small amounts of platinum from the electrodes had reacted with NH₄Cl to produce various platinum amine halide complexes. Two of these, *cis*-[Pt(NH₃)₂Cl₂] and its corresponding tetrachloro complex *cis*- [Pt(NH₃)₂Cl₄] were capable of inducing filamentous growth in the absence of an electric field [1]. It has been known that this kind of growth indicates the potential antitumor activity of the corresponding chemicals. Therefore, Rosenberg and coworkers performed experiments with sarcoma 180 and leukemia L1210 bearing mice [2]. This led to cisplatin entering phase I clinical trials in 1971. Approval of cisplatin for treatment of testicular and ovarian cancer was given in 1978 [4]. Currently, cisplatin is one of the most widely used antitumor drugs. It is highly effective in treating testicular, ovarian, bladder, cervical, head and neck, and small-cell and non-small cell lung cancers [3]. Despite its activity in many cancers, cisplatin is ineffective in others e.g. leukemia, renal and gastrointestinal cancers. The major barrier to cisplatin efficacy is perhaps the drug resistance, which can be either intrinsic or acquired [3]. That means for the latter case that many cancers, including ovarian cancer, initially responsive to cisplatin become resistant to it. It also has major toxicity limitations of which nephrotoxicity is the most notable, although nausea and vomiting, peripheral neuropathy, and myelotoxicity can also raise major concerns [4]. Cellular resistance to cisplatin is due to several factors and has been reviewed in details [5]. For cisplatin, which is routinely used in dosages at the limit of its systemic toxicity, the level of resistance can completely eliminate clinical effectiveness [5]. The major causes of resistance that have been observed are the prevention of sufficient amount of drug from reaching and binding to DNA and a failure of cell death which taking place after binding of Pt to DNA [6]. Reduced platinum accumulation and increased cytoplasmic...
detoxification by glutathione and or metallothioneins represent the major causes of inadequate drug concentrations reaching DNA. Once DNA binding has occurred, resistance mechanisms include increased DNA repair of adducts, and an ability to tolerate greater levels of DNA damage with concomitant failure to engage programmed cell death (apoptotic) pathways. Elucidation of these mechanisms of resistance has been essential in providing a basis for the development of Pt-based complexes capable of circumventing cisplatin resistance.

Since the introduction of cisplatin, thousands of platinum complexes have been synthesized and evaluated for their antitumor activity. The main aim of these intensive investigations was to obtain drugs with at least an equal activity but reduced toxicity compared to cisplatin. The strategy to reduce toxicity involved increasing the solubility in water and stability of the complexes. This has been generally achieved by replacing the chloro ligands either with chelating carboxylate, oxalate, sulfate or glycolate. This kind of leaving group is the main feature of the second generation compounds. The most successful of them is carboplatin [(NH₃)₂Pt(C₄H₆O₄)] (Figure 1. III. 1). It has improved the therapeutic index of cisplatin by ameliorating some of the toxic side effects [7]. Although it has a lower activity than cisplatin, its decreased toxicity allows outpatient administration without need for forced diuresis and very high dosages (up to 2000 mg/dose) can be achieved with possibly more antitumor efficacy [8]. Nevertheless, it appears to have the same spectrum of anticancer activity of cisplatin and thus is not active against cisplatin resistant tumors [9]. Several carboplatin analogue compounds containing cyclobutane ring were developed in the late 80s, in the hope of improving the carboplatin characteristics. The compounds include zeniplatin (CL 286558), [1,1'-cyclobutanedi-carboxylato{2,2'-bis(aminomethyl)-1,3-propandiol} -platinum(II)]; Enloplatin (CL 287110) [1,1'-cyclobutane-dicarboxylatetetrahydro-4Hpyprazine-4,4-dimethylamineplatinum(II)]; Miboplatin (DWA 2114R), [R(-)-1,1'-cyclobutanedicarboxylato-2-aminomethylpyrrolidine-platinum(II)] and CI-973 (NK 121), [1,1'-cyclobutanedicarboxylato-2-methyl-1,4-butandiamineplatinum(II)] [10]. Other compounds that were developed later but are still linked to the second generation compounds through their structures include Lobaplatin, (D-19466), [lactatodiaminomethylcyclobutaneplatinum(II)], SKI 2053 R[malonatomethyl
isopropyldimethylaminodioxelane)platinum(II)], Cytoplatam [malatoaminecyclopentylamine) platinum(II)], and Nedaplatin (254-S), [cis-glycolatodiamine-platinum(II)] [10]. From the second generation, only carboplatin fulfilled all the requirements for marketing approval worldwide, while Lobaplatin (Figure 1. III. 1) which was introduced into clinical trials in 1992 for cisplatin-resistant ovarian cancer, head and neck cancers, and small-cell lung cancer, has been approved in China for the treatment of breast cancer and Nedaplatin received approval for use in Japan in 1998 [10]. As a single agent in phase II studies, response rates of about 25% were observed for head and neck, testicular, lung, bladder, ovarian, and cervical cancer. The compounds Zeniplatin, Enloplatin, Miboplatin and CI-973 have all been abandoned due to insufficient activity or unacceptable side effects.

A second generation analogue of cisplatin, carboplatin has reduced toxic side effects for the same efficiency thanks to its much lower reactivity. Unfortunately, carboplatin is only active in the same range of tumors as cisplatin and still administrated intravenously. The third generation of drugs includes compounds that contain different types of chiral amines [10, 11]. Oxaliplatin (Figure 1. III. 1) (cis-oxalato-trans-l,1,2-diaminocyclohexaneplatinum(II)) showed antitumor activity in colorectal cancer [12, 13], had positive preclinical evaluations for use in cisplatin resistant tumors and can be administrated orally. Investigations on these types of chiral complexes showed that the trans isomer trans-l (trans-(−)-1R,2R) is more efficacious than the corresponding trans-d- (trans-(+)−1S,2S) and the cis-isomer (1R,2S) [14]. Thus, the activity might be explained by speculating on the stereochemical structures of the complexes.

Recently, some pioneering strategies towards the synthesis of novel platinum anticancer drugs have emerged [15, 16]. Those are based on changing the coordinated nitrogen ligand or altering the leaving groups. Other strategies have focused on changing the type of the metal center (e.g. palladium (II) complexes) or applying platinum(IV) complexes that are relatively more soluble in water. Attention also has been shifted to discover "nonclassical" drugs that can act in a manner different from cisplatin [17]. Unconventional structures that violate the empirical structure-activity rules (SAR) of platinum compounds lacking NH, NH₂, or NH₃ ligands and multinuclear complexes are
examples of these compounds that are designed to circumvent cisplatin resistance and enhance its activity.

Water insolubility and low bioavailability prevent cisplatin from being an orally active drug [18]. Therefore, a new class of Pt(IV) compounds has been developed to get increased solubility in water. These drugs could represent a clinical advantage in terms of ease administration, particularly in patients who could not be treated systematically and allow the possibility of treatment on an outpatient basis, thus substantially reducing hospitalization costs. These compounds are typically neutral, water soluble, and robust enough to survive the gastric environment. Platinum(IV) complexes are known to be much more tolerant to ligand substitution reactions than their Pt(II) counterparts [19]. In order to rationally design new Pt(IV) complexes, correlation between structure, reduction and activity were needed, since it is generally admitted that Pt(IV) compounds must be reduced to be activated. Hambley et al. showed that the potentials for the reduction of Pt(IV) to Pt(II) depend on the nature of the axial ligand for a series of ethylenediamine-based Pt(IV) complexes. Reduction occurs most readily when the axial ligands are Cl > OCOR > OH [19]. In another study, Choi et al. showed that the reduction rates which correlate with the reduction potentials depend on the electron-withdrawing influence of the axial ligands and the steric hindrance of axial and carrier ligand [20]. For the studied ethylenediamine-based Pt(IV) complexes the fastest reduction rate (OH < OCOCH$_3$ < Cl < OCOCF$_3$) corresponds to the most electron-withdrawing axial ligand and coincides with the highest cytotoxicity. As Pt(IV) complexes need to be reduced to become active, if illumination could increase in and around the tumor the rate of reduction of Pt(IV) to Pt(II), then a more effective and less toxic therapy could be achieved. The development of Pt(IV) prodrugs that can be photoreduced to cytotoxic Pt(II) species by visible light has been pursued by Sadler and coworkers, who synthesized the *trans, cis*-[(OCOCH$_3$)$_2$(en)PtI$_2$] [21]. The toxicity of this complex towards human cancer cells is enhanced by 35% when the treated cells are irradiated with light of $\lambda > 375$ nm. Other non-toxic, inert photoactive Pt(IV) cisplatin analogues, which after administration might remain inactive until selectively irradiated at target site were designed. Examples include the photoactive diazido complexes, *cis, trans, cis*- [Pt(N$_3$)$_2$(OH)$_2$(NH$_3$)$_2$] and *cis, trans*- [Pt(en)(N$_3$)$_2$(OH)$_2$], which can be activated by UV or
visible light to Pt(II) species with the loss of the two azide ligands, and have been shown to bind to guanine on photoactivation [22]. Despite that Kelland and coworkers have found that dicarboxylate Pt(IV) complexes of the general formula cis, trans, cis-[(OCOR₁)(NH₃)(RNH₂)Pt(IV)Cl₂] (R, R₁: alkyl groups) are up to 840-fold more active than cisplatin in in-vitro assays, none of the Pt(IV) compounds have revealed significantly greater activity in humans. Reduction of many Pt(IV) complexes, with loss of the axial ligands, occurs rapidly in vivo and the consequent loss of lipophilicity probably accounts for the non translation of the in vitro activity to animal systems. Some Pt(IV) complexes have shown sufficient promise to enter clinical trials: Iproplatin (CHIP, JM9, cis,trans,cis-[(isopropylamine)₂Pt(Cl₂)(OH)₂], Tetraplatin or ormaplatin [(d,l-cyclohexane-1,2-diamine)PtCl₄], and Satraplatin (JM216) cis,trans-[PtCl₂(OAc)₂(NH₃)(cyclohexasmine)] [10].

Iproplatin was sufficiently well tolerated to enter phase II and III clinical trials but found to be less active than cisplatin and so has not entered widespread clinical use. Tetraplatin has also been abandoned at the phase I level because it caused severe neurotoxicity in treated patients despite that it exhibits a broad spectrum of antitumor activity and is also active against cisplatin resistant cell lines. Satraplatin showed superior activity compared to cisplatin against human cervical, small-cell lung, and ovarian carcinoma cell lines [23]. It entered phase III trials, but the trials were abandoned due to variability in drug uptake.

The discovery of several trans-Pt complexes with in vitro and in vivo activity against tumor cells resistant to cisplatin has forced the re-evaluation of the structure-activity relationships (SAR) for platinum antitumor agents [24]. For the reason that the factors that influence the cytotoxic activity of trans-Pt complexes do not follow the same patterns as those found for cisplatin and its analogues, the differences in cellular and molecular pharmacology between trans-Pt complexes and cisplatin could be systematically exploited to design novel trans platinum complexes with a clinical profile complementary to that of cisplatin and related analogues. While isomerization of the trans compounds to an active cis isomer could account for some activity of the trans isomer, in many cases cis isomers are less active than the corresponding trans isomers. Transplatin (Figure 1. III. 1) is kinetically more reactive than cisplatin and more susceptible to deactivation [25]. Careful design applying sterically hindered ligands may
reduce kinetic reactivity of the trans isomers of platinum complexes. As the trans isomer forms different Pt-DNA adducts than cisplatin analogues, it is hoped that trans platinum complexes could overcome cisplatin resistance in certain tumors. Farrell et al. reported that the presence of a planar ligand such as pyridine or quinoline greatly enhances the cytotoxicity of the trans isomer, so that cytotoxicity is equivalent to cisplatin itself. As expected, the cytotoxicity of trans complexes containing planar ligands is highlighted by a remarkably low resistance factor in murine and human cisplatin resistance tumor cell lines. Studies on the DNA interaction of trans-[(NH₃)(quinoline)PtCl₂] reveal that this complex forms considerably more interstrand cross-links than transplatin. In addition to this higher cross-linking efficiency, the quinoline ligand can interact with the duplex, which could induce specific conformational alterations around the site of platination and influence in protein recognition. Natile et al. reported that the trans platinum-iminoether complexes can show higher activity than the corresponding cis isomers. They also reported that the E or Z configurations of iminoether ligand affect the activity of the platinum complexes [26]. The greater lipophilicity of the E isomer determines a greater cellular accumulation and in vitro cytotoxicity than the Z isomer. On the contrary, in the in vivo P388 leukemia system, the Z isomer appears to be more active than the E isomer, and the possible pharmacokinetic reasons for this behavior are at present under investigation. Binding to DNA of these cationic trans-Pt complexes might not need as a pre-requisite for the formation of platinum aqua species. Hence, there would be an electrostatically driven pre-association process between the positive charge of the trans-Pt compound and DNA phosphates prior to coordination of the trans-Pt center to N(7) of guanine or adinine [27]. Also Kelland et al. reported that a trans-platinum complex exhibited greater in vitro cytotoxicity against human carcinoma cell lines than its corresponding cis isomer. The complex is a Pt(IV) species, trans, trans, trans-[amine(cyclohexylamine)Pt(IV) dichloride], (JM335). The platinum(II) counterpart of the complex (JM334) did not show in vivo antitumor activity. Positively charged, water solubile cis- and trans-[PtCl₂(piperazine)(amine)] complexes (amine = NH₃, n-butylamine, isopropylamine, 4-picoline, piperidine, and piperazine) were reported to have significant cytotoxic activity against cisplatin resistant ovarian cancer cells [28, 29]. The charged complexes are taken up by cancer cells much more rapidly than cisplatin and
bind to cellular DNA and to calf thymus DNA much faster than cisplatin or transplatin [10]. The results reported suggest that combination of positively charged ligands with a *trans*-[Pt(II)Cl$_2$] species may lead to a new family of platinum tumor agents that are able to circumvent cisplatin resistance.

(1) Cisplatin

(2) Transplatin

(3) Carboplatin

(4) Nedaplatin

(5) Oxaliplatin

(6) Lobaplatin

(7) ZDO0473

(8) JM216

(9) Spiroplatin

(10) Sulphato(1,2-diaminocyclohexane) platinum(II)
Figure 1. III. 1 Structures of some antitumour platinum complexes
References
Section IV
Objectives and importance of the present work

As seen from section III, the development of new Pt-containing drugs always continues unabated. There are still questions concerning the mechanism of activation, appearance of resistance during therapy, enlargement of the field of application, etc., unsolved. Binding interactions of the chemotherapeutic agent like cisplatin and its structural analogues with DNA and its fragments i.e. amino acids, nucleosides, and nucleotides are being recently investigated thoroughly. Although intensive efforts of various research groups in recent past have contributed significantly towards understanding of the antitumour activity of platinum complexes, most of the studies focused on the structural identification of the binding of the DNA constituents to the metal centre using NMR, HPLC, ESI-MS and X-ray technique. Significantly very less is known about the mechanistic details with regard to the various steps of adduct formation and the factors controlling such adduct formation. Understanding of contrasting biological activities of different metallo-drugs requires insight not only into the structures of their adducts with biomolecules, but also into the reaction pathway and the formation of intermediates. This involves investigations on substitution of metal complexes with small and large biomolecules. The precise mechanism remains elusive, cisplatin and its several analogues have provided a fertile ground for exciting (bio) chemistry. This thesis work will deal with a special aspect of platinum chemistry, namely, its reactivity with a group of ligands that is not present in the drug but nevertheless must play a key role in the process of drug distribution in the body, in the mechanism of metabolism of the Pt(II) antitumor compounds, in the therapeutic effect, and in the serious toxic side effects of cisplatin. Therefore mechanistic knowledge of reaction of cis-(N-N)-chelated platinum(II) diaqua complexes with amino acids, substituted amino acids, dipeptides, nucleosides and other biomolecules may be useful to design metal complexes as chemotherapeutic agents for recognizing new targets under in vivo condition. To gain more insight into the interaction of Pt(II) complexes with N-, O- and S-donors, the following nucleophiles were chosen for investigation throughout this work (CHAPTER 2-7): adenosine (model for nucleobase binding); N,N′-diethylthiourea (rescue agent); L-asparagine, L-arginine L-glutamic acid and DL-penicillamine (amino acid or substituted amino acid); glycyl-L-leucine (dipeptide); 2-thiouracil, 1,2-cyclohexanedionedioxime and acetylaceitone (ligands with different donor
atoms; model for competitive study). The selected nucleophiles are biologically relevant with different electronic or structural properties. This type of interaction study provides new clues for explaining the chemistry, bio-chemistry and cellular action of cisplatin. It helps to design new generation platinum based drugs as well as to understand the ligand exchange and DNA-platination reactions. For these purposes complexes \(\text{cis-}[\text{Pt}(\text{cis-dach})(\text{OH}_2)_2]^{2+}\) and \(\text{cis-}[\text{Pt}((\text{en})(\text{OH}_2)_2]^{2+}\) were chosen as model antitumour compounds. The aqua series \(\text{cis-}[\text{Pt}((\text{NH}_3)_2(\text{OH}_2)_2]^{2+}\) react much faster with nucleic acids than the dichloro series [1]. The complex, \(\text{cis-}[\text{Pt}(\text{cis-dach})(\text{OH}_2)_2]^{2+}\) has excellent antitumour activity with little or no nephrotoxicity and cross resistance with DDP [2]. The hydroxobridged dimers and trimers of dach-Pt(II) complexes are active anticancer agent and are less toxic than monomer in contrast to the \(\text{NH}_3\) oligomers of cisplatin [3].

The prime concern of the work is the study of interactions of platinum complex(es) with some ligands at different [complex], [ligand], pH, solvents and temperatures. These ligands will compete with the DNA for the reaction with any antitumour agent. Therefore, it is of biological significance to evaluate the equilibrium constants for the displacement reaction. These equilibrium constants may give a measure of the effectiveness of the antitumour agent. Much emphasis will be given to the temperature dependence of the rate constants which give the activation parameters. From the experimental findings a plausible mechanism will be proposed.

In summarized form the aims of this work were to:

1. synthesise model compounds which are established anticancer drugs.
2. observe the kinetic effect and deduce the mechanism of the square-planar substitution reactions of these complexes with different ligands, which have biological significance.
3. determine the effect of nucleophiles on the reaction kinetics in order to investigate how electronic and steric factors within the metal complex affect its reactivity.
4. use the conclusions from the study to help in the future designing of new types of anti-cancer drugs.

In conclusion, this work could clearly demonstrate a variety of options of how to modify the reactivity of Pt(II) complexes via \(\sigma\)-donor effects. These findings may contribute to the toolbox of the synthetic chemist to choose from in the design of new anti-tumor drugs.
References


Section V

Instrumental techniques and general information about the chemicals

1. Instruments used
   i. **Elemental analyses**: Perkin-Elmer 240 CHNS/O analyser.
   ii. **Infrared Spectra**: Perkin-Elmer FTIR model RX1 Infrared spectrophotometer.
   iii. **Electronic Spectra**: Shimadzu spectrophotometer (UV-2101PC) equipped with a Shimadzu TB 85 thermobath (accuracy = ± 0.1°C) and also with a Shimadzu UV–Vis spectrophotometer (UV-2450), attached to a thermoelectric cell temperature controller (model TCC-240A with an accuracy of ± 0.1 °C).
   iv. **pH**: Systronics digital pH meter (model 335) with an accuracy of ± 0.01 unit and Sartorius Digital pH meter (model PB11) with an accuracy of ± 0.01 units.
   v. **ESI-MS**: ESI-mass spectra recorded using a micromass Q-Tof micro™ mass spectrometer in +ve ion mode.
   vi. **Conductance**: Systronics conductivity meter model 308.
   vii. **¹H NMR**: Bruker AVANCE III 400 MHz spectrometer.

2. **Chemicals and Solvents used**: A.R. grade chemicals were procured from renowned companies like E. Merck, Fluka, Aldrich and used without further purification. Doubly distilled/milli Q water was used to prepare all the solutions.
Chapter 2

Kinetics and mechanism of the interaction of DL-penicillamine with cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) perchlorate in aqueous medium
2.1 Introduction

Substitution on square planar platinum(II) complexes is of considerable interest in chemical as well as in bio-medical research. In particular, cisplatin [1-7] and its structural analogues [8] are widely used in the treatment of specific cancers. It is now accepted that these platinum (II) complexes exercise their anti-tumor activity by inhibiting the replication of cellular DNA [9-10]. Nephrotoxicity and neurotoxicity coupled with drug resistance, which develops within the patients after initial treatment have limited the wider clinical application [11] of chloro derivatives of (N, N) - chelated Pt(II) complexes. Therefore, the search for other drugs has continued and the aqua variety was found to be superior to some extent in this respect as the coordinated water molecule is better leaving group than the chloro ligand in Pt(II) complexes [6]. In this context the diaqua derivative of cis-[Pt(cis-dach)Cl2] known as an effective anti-tumor compound with little nephrotoxicity and has cross resistance with DDP [12]. The hydroxobridged dimer and trimer of dach-Pt(II) complexes are active anti cancer agent and are less toxic than the monomer in contrast to NH3 oligomers.

Pt(II) complexes can also interact with many other bio-molecules, especially those containing sulfur for which Pt(II) has a very high affinity. Interaction of platinum(II) complexes with sulfur containing bio-molecules are responsible for a variety of biological effects, such as inactivation of Pt(II) complexes, development of cellular resistance to platinum, and toxic side effect such as nephrotoxicity [13-14]. The reactivity of aqua amine complexes of Pt(II) towards ‘S’ containing amino acids and ‘S’ containing substituted amino acids is thereof interest. In this chapter, the detailed kinetic and mechanistic studies of aqua ligand substitution from cis-[Pt(cis-dach)(OH2)2]2+ by DL-penicillamine (β, β’-dimethyl cysteine; a degradation product of β-lactum antibiotic, penicilline and also a ‘S’ containing bio-active ligand) in aqueous medium have been described.

2.2 Experimental

The chloro complex cis-dichloro-(cis-dach)platinum(II) was prepared according to literature method [15]. The reactant complex cis-[Pt(cis-dach)(OH2)2](ClO4)2 (I) (dach = diaminocyclohexane) was prepared from cis-dichloro(cis-dach)platinum(II) by hydrolysis in the presence of two molar equivalent of silver perchlorate. The chloro
compound sprinkled over an aqueous solution of silver perchlorate. The suspension was kept in dark for more than 24 hours and then filtered to remove AgCl. The precipitate was washed with water and the volume of the solution was made up to the mark. The diaqua complex solution was then characterized by spectrophotometrically. The pH of the solution was maintained at pH (4.0), so that perchlorate salt exists as the diaqua species. The product of the reaction between (I) and DL-penicillamine was prepared by mixing the reagents at pH 4.0 in different molar ratio: viz 1:1, 1:2, 1:3, 1:4 and 1:5 and thermo stating the mixture at 60 °C for 48 hours. The absorption spectra of the resulting solution were recorded and all were found to exhibit almost identical absorbances at the same wavelength at 240 nm. The spectral difference between the product complex and the substrate complex is shown in Figure 2.1.

![Figure 2.1: Spectral difference between complex (I) and the DL-penicillamine substituted product; (1) [complex (I)] = 2.00 × 10^{-4} \text{ mol dm}^{-3}; (2) [complex (I)] = 2.00 \times 10^{-4} \text{ mol dm}^{-3}; [DL-penicillamine] = 4.00 \times 10^{-3} \text{ mol dm}^{-3}, cell used = 1 \text{ cm quartz, pH = 4.0, and ionic strength = 0.1mol dm}^{-3} \text{ NaClO}_4.](image)

The composition of the product in the reaction mixture was determined by Job’s method of continuous variation (Figure 2.2). The metal: ligand ratio was found to be 1:1.
Figure 2.2: Job’s plot for the reaction between complex (I) and DL-penicillamine at pH = 4.0 and ionic strength = 0.1 mol dm^{-3} NaClO₄.

Cis-[Pt(cis-dach)(OH₂)]²⁺ and DL-penicillamine were mixed in 1:1 molar ratio at pH 4.0 and a yellow solid was isolated. The IR spectrum of the yellow product in the KBr disc shows strong bands at 3428 and 510 cm⁻¹ together with prominent bands at 1711, 1627 and 1406 cm⁻¹. The asymmetric COO⁻ stretching frequency (ν_{asym}) of the amino acids occurs at 1580-1660 cm⁻¹ when the group is coordinated to metals, where as a non-coordinated COO⁻ group has the ν_{asym}(COO⁻) stretching at lower frequency [16]. The band at 1711 cm⁻¹ is therefore due to the ν_{asym}(COOH) of the free carboxyl group. The band at 1627 cm⁻¹ may be due to overlapping of the ν (asym) COO⁻ and δ NH₂ bending motion coordinated to platinum, indicating that the COOH group is not a ligation site. The presence of a strong –OH stretching band at 3428 cm⁻¹ indicate the presence of free carboxyl (-COOH) group. The absence of weak absorption of the –SH group at 2500 cm⁻¹, present in free DL-penicillamine, indicate formation of the Pt-S bond in the product complex [17]. The band at 510 cm⁻¹ is also assigned to ν (Pt-N) bond formation [18]. The spectrum suggests that the final product is an (S, N) coordinated chelate and the DL-penicillamine behaves as a bidentate ligand in the experimental pH. The observation is consistent with other studies where (N, N)-platinum complexes bind to S-containing amino acids, substituted amino acids and dipeptides through N and S as has been described previously [19-21].

The conductance measurement also reveals that bonding occurs through S and N end of the DL-penicillamine ligand. During chelation, one proton is released from the –
SH group of DL-penicillamine which is supported by the increase in conductance with the reaction. Affinity of platinum(II) for sulfur is high that provides the driving force for the deprotonation in the first step.

The aqueous solution of cis-[Pt(cis-dach)(OH$_2$)$_2$]$^{2+}$ and DL-penicillamine were mixed in a 1:1 molar ratio and the mixture was thermo stated at 60 °C for 48 hours and used for ESI-MS measurement. The ESI mass spectrum of the resulting solution is shown in Figure 2.3.
Figure 2.3: ESI-mass spectrum of the product for complex (I) and the DL-penicillamine substituted product at pH 4.0 in aqueous medium and at ionic strength 0.1 mol dm$^{-3}$ NaClO$_4$. 
It is clear from this spectrum that the ion at m/z 457.34 (minor peak) has become the precursor ion species in the mixture solution and this is tentatively attributed to (DL-penicillamine + Pt + dach) \(^+\); the relative abundance of isotope peaks match the expected values, i.e., m/z 456.01, m/z 457.34, m/z 458.52. The fragment ion at m/z 413.15 corresponds to loss of 44 u from the precursor ion and is thus attributed to loss of CO\(_2\), at the same time, the peak at 414.09 is the isotopic peak for the loss of CO\(_2\). The fragment ion at m/z 318.12 has the maximum relative abundance as expected for [Pt+ 2 NH\(_3\) + S-CH (CH\(_3\))-CH\(_2\)-NH\(_2\)]\(^+\) and peak at 319.16 is the isotopic peak for the same. The precursor ion and the fragmented products are shown schematically in Figure 2.4.

![Schematic diagram of ions](image)

**Figure 2.4:** Plausible structures of the precursor ions and the fragmented ions matched with their m/z values obtained from the ESI-mass spectrum.

Pt(II) is likely to be coordinated to both the sulfur atom and the NH\(_2\) group considering the strong coordination ability of these functional groups [22-24]. Thus the structure proposed here for product ion specie, deduced from ESI-mass spectrum, is generally consistent with those derived from other experimental methods.

### 2.3 Physical measurement

The kinetic studies were done on a Shimadzu UV 2101 PC spectrophotometer equipped with a Shimadzu TB 85 thermobath (accuracy ± 0.1 °C). IR Spectra (KBr disc, 4000 –
300 cm\(^{-1}\)) were measured in Perkin-Elmer FTIR model RX1 Infrared spectrophotometer. ESI-mass spectra recorded using a micromass Q-Tof micro\(^{\text{TM}}\) mass spectrometer in +ve ion mode. Conductance measurements were carried out in a Systronics conductivity meter model 308 where the cell constant was calibrated with 0.01 mol L\(^{-1}\) KCl solution and water used as solvent. The pHs of the solutions were adjusted by adding NaOH/HClO\(_4\) and the measurements were carried out with the help of a Sartorius Digital pH meter (model PB11) with an accuracy of ± 0.01 units. Doubly distilled water was used to prepare all the solutions. All other chemicals used were of AR grade. The reactions were carried out at constant ionic strength (0.1 mol dm\(^{-3}\) NaClO\(_4\)).

2.4 Kinetic measurements

Kinetic measurements were carried out on a Shimadzu spectrophotometer (UV-2101 PC) equipped with a Shimadzu TB 85 thermo bath (accuracy ±0.1 °C). The absorption due to DL-penicillamine was subtracted by using 1:1 (molar ratio) ligand: water mixture in the reference cell. The absorbance at 240 nm was monitored to follow the progress of the reaction. Conventional mixing technique was followed and pseudo first order conditions with respect to metal ion concentration, were maintained throughout the course of the reaction. A typical plot of ln(A\(_{\infty}\) - A\(_{t}\)) (where A\(_t\) and A\(_{\infty}\) are absorbances at time (t) and after completion of the reaction) against time (t) (Figure 2.5) is found to be nonlinear; it is curved at initial stage and subsequently of constant slope indicating that the reaction proceeds via two consecutive steps. From the limiting linear portion of ln (A\(_{\infty}\) - A\(_{t}\)) versus time (t) curve, k\(_2\) (obs) was obtained. The k\(_1\) (obs) values were obtained from the plot of ln\(\Delta\) versus time (t) where time (t) is small (Figure 2.6). Origin software was used for computational works. Rate data, represented as an average of duplicate runs, are reproducible within ± 4%.
Figure 2.5: A typical kinetic plot of $\ln(A_\infty - A_t)$ versus time ($t$). [complex (I)] = $2 \times 10^{-4}$ mol dm$^{-3}$; [DL-Penicillamine] = $4 \times 10^{-3}$ mol dm$^{-3}$; pH = 4.0; $\mu$ = 0.1 mol dm$^{-3}$ NaClO$_4$ and Temperature = 45 °C.

Figure 2.6: A typical kinetic plot of $\ln\Delta$ versus time ($t$). [complex (I)] = $2.0 \times 10^{-4}$ mol dm$^{-3}$; [DL-Penicillamine] = $4.0 \times 10^{-3}$ mol dm$^{-3}$; pH = 4.0; $\mu$ = 0.1 mol dm$^{-3}$ NaClO$_4$ and Temperature = 45 °C.

2.5 Results and discussion

The pK$_1$, pK$_2$ and pK$_3$ of DL-penicillamine [25] are 1.90, 7.88 and 10.58 at 25 °C respectively, which refer to following dissociation processes:
Scheme 2.1. Acid dissociation equilibria of the ligand DL-penicillamine

So at pH 4.0 the major species involved in the kinetic process is zwitterionic form (II) of DL-penicillamine. The pK₁ and pK₂ (6.25 and 7.80) for cis-(diaqua(cis-1,2-diaminocyclohexane)platinum(II) have been evaluated by Irving–Rossotti titration technique [26]. It can be assumed that at pH 4.0 the reactant exists as the diaqua ion.

The reaction involves a two-step consecutive process; the first step is dependent on ligand concentration whereas the second one is independent. In the first step one aqua ligand was replaced from cis-[Pt(cis-dach)(OH₂)₂]²⁺ by DL-penicillamine and in the slower step, another aqua ligand is substituted. This is the ring closure step. The rate constant for such a process can be evaluated by assuming the following scheme:

\[
A \xrightarrow{k_1} B \xrightarrow{k_2} C
\]

Where A is the diaqua species (I); B is the single substituted intermediate and C is the final product (2) [Pt(cis-dach)(DL-penicillamine)]⁺. Formation of C from B is predominant after some time has elapsed.

2.6 Calculation of k₁ for A→B step

The rate constant k₁(obs) for the A→B step can be evaluated by the method of Weyh and Hamm [27] using the usual consecutive rate law;
\[(A_\infty - A_t) = a_1 \exp (-k_{1\text{(obs)}} t) + a_2 \exp (-k_{2\text{(obs)}} t) \]  \hspace{0.5cm} (2.1)

Whence
\[(A_\infty - A_t) - a_2 \exp (-k_{2\text{(obs)}} t) = a_1 \exp (-k_{1\text{(obs)}} t) \]  \hspace{0.5cm} (2.2)

Where \(a_1\) and \(a_2\) are constants dependent upon the rate constant and extinction coefficient. Values of \((A_\infty - A_t) - a_2 \exp (-k_{2\text{(obs)}} t)\) are obtained from X-Y at different time (t), so that
\[\Delta = a_1 \exp (-k_{1\text{(obs)}} t)\]

or,
\[
\ln \Delta = \text{constant} - k_{1\text{(obs)}} t \]  \hspace{0.5cm} (2.3)

Where \(\Delta\) is the difference of \(\ln(A_\infty - A_t)\) values between the observed and extrapolated part of the linear portion of \(\ln(A_\infty - A_t)\) versus time (t) curve at any time (t). \(k_{1\text{(obs)}}\) is derived from the slope of \(\ln\Delta\) versus time(t) plot (Correlation Coefficient 0.998), when time (t) is small (Figure 2.6).

A similar procedure is applied for DL-penicillamine in the concentration range 0.002 – 0.006 mol dm\(^{-3}\) at constant complex (I) concentration of 0.0002 mol dm\(^{-3}\) at pH 4.0 and at 35, 40, 45, 50 and 55 °C respectively. The \(k_{1\text{(obs)}}\) values thus obtained are linearly dependent on the studied concentration range. The \(k_{1\text{(obs)}}\) values for different ligand concentrations at different temperatures are given in Table 2.1.

**Table 2.1**: \(10^3\) \(k_{1\text{(obs)}}\) (s\(^{-1}\)) values for different concentration of DL-penicillamine at different temperatures. [Complex (I)] = 2 \(\times 10^{-4}\) mol dm\(^{-3}\), pH = 4.0, \(\mu = 0.1\) mol dm\(^{-3}\) NaClO\(_4\)

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>(10^3) [DL-Penicillamine] (mol dm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.00</td>
</tr>
<tr>
<td>35</td>
<td>0.62</td>
</tr>
<tr>
<td>40</td>
<td>0.93</td>
</tr>
<tr>
<td>45</td>
<td>1.48</td>
</tr>
<tr>
<td>50</td>
<td>1.97</td>
</tr>
<tr>
<td>55</td>
<td>3.27</td>
</tr>
</tbody>
</table>

The ligand concentration dependence of \(k_{1\text{(obs)}}\) values can be explained in terms of rapid formation of an outer sphere association complex between the reactant complex (I) and
the sulfur end of zwitterionic form of ligand in the A → B stage. The rate increases with increase in ligand concentration and reaches a limiting value (Figure 2.7), which is probable due to the completion of the outer sphere association complex formation. Since the metal ion reacts with immediate environment, further change in ligand concentration beyond the saturation point will not affect the reaction rate and a gradual approach towards limiting rate is observed. At this stage the interchange of the ligands from outer sphere to the inner sphere occurs. The following scheme 2.2 can be proposed:

\[
\begin{align*}
[\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_2]^{2+} + \text{L-LH} & \xrightleftharpoons[K_E]{k_1} [\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_2]^{2+} \ldots \text{L-LH} \\
\text{A} & \quad \text{Outer sphere association complex} \\
[\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_2]^{2+} \ldots \text{L-LH} & \xrightarrow[k_2]{\text{chelation}} [\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})(\text{L-L})]^+ + \text{H}_3\text{O}^+ \\
\text{B} & \\
[\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})(\text{L-L})]^+ & \xrightarrow[k_2]{\text{chelation}} [\text{Pt}(\text{cis-dach})(\text{L-L})]^+ + \text{H}_2\text{O} \\
\text{B} & 
\end{align*}
\]

\textbf{Scheme 2.2}

Where L-LH is the zwitterionic form of DL-penicillamine.

Based on the above scheme, a rate expression (2.7) can be derived for the A → B step:

\[
\frac{\text{d}B}{\text{d}t} = k_1 K_E [\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_2]^{2+}_{\text{total}} [\text{DL-penicillamine}] / 1 + K_E [\text{DL-penicillamine}] 
\]

or,

\[
\frac{\text{d}B}{\text{d}t} = k_{1(\text{obs})} [\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_2]^{2+}_{\text{total}} 
\]

Where [\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_2]^{2+}_{\text{total}} is the concentration of the unreacted complex, [DL-penicillamine] is the concentration of DL-penicillamine. Hence it can be written;

\[
k_{1(\text{obs})} = k_1 K_E [\text{DL-penicillamine}] / 1 + K_E [\text{DL-penicillamine}] 
\]

Where \(k_1\) is the rate constant for conversion of outer sphere complex to inner sphere complex and \(K_E\) is the outer sphere association equilibrium constant. Equation (2.6) can be rearranged:

\[
1 / k_{1(\text{obs})} = 1 / k_1 + 1 / k_1 K_E [\text{DL-penicillamine}] 
\]

A plot of 1 / \(k_{1(\text{obs})}\) versus 1 / [DL-penicillamine] should be linear with an intercept of 1/k_1 and slope 1 / k_1K_E. This was found to be so, at all temperature studied (Figure 2.8), the k_1 and K_E values obtained from intercept and from slope to intercept ratio, respectively and are included in Table 2.2.
2.7 Calculation of $k_2$ for the B→C step

The B→C step is the ring closure step in which the amino group of the substituted amino acid, DL-penicillamine binds the metal centre. Due to the steric hindrance, this chelation step is slower and independent of ligand concentration variation. At each temperature, the $k_2$ values were calculated from the limiting linear portion (where time $t$ is large) of the $\ln(A_\infty - A_t)$ versus time $t$ curves (Figure 2.5) and are given in Table 2.2.

**Table 2.2:** $k_1$, $k_2$ and $K_E$ values for the substitution reaction

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$10^3 k_1$ (s⁻¹)</th>
<th>$10^5 k_2$ (s⁻¹)</th>
<th>$K_E$ (dm³ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>3.26</td>
<td>2.56</td>
<td>117</td>
</tr>
<tr>
<td>40</td>
<td>4.59</td>
<td>3.37</td>
<td>127</td>
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<tr>
<td>45</td>
<td>5.89</td>
<td>4.54</td>
<td>167</td>
</tr>
<tr>
<td>50</td>
<td>6.35</td>
<td>6.32</td>
<td>227</td>
</tr>
<tr>
<td>55</td>
<td>8.75</td>
<td>7.77</td>
<td>297</td>
</tr>
</tbody>
</table>

2.8 Effect of pH on reaction rate

The reaction was studied at four different pH values. The $k_{obs}$ values are found to increase with increase in pH in the studied pH range. At a fixed 0.0002 mol dm⁻³ [complex($J$)], 0.004 mol dm⁻³ [DL-penicillamine] and 0.1 mol dm⁻³ ionic strength the $10^3k_{1(obs)}$ values at 50 °C in aqueous medium are 2.60, 2.86, 3.01 and 3.66 s⁻¹ and $10^5k_{2(obs)}$ values are 4.61, 5.27, 6.32 and 7.66 s⁻¹ at pH 2.8, 3.4, 4.0 and 4.6 respectively.
The enhancement in rate may be explained based on two acid dissociation equilibria of the ligand and the complex. In the studied pH range (pH 2.8 to 4.6) with increase in pH, the diaqua complex will be converted into hydroxoaqua complex. The reactivity of hydroxoaqua complex is usually higher than that of diaqua complex by the well-known labilising effect of the coordinated hydroxide ion. At the same time, with increase in pH deprotonation of the ligand occurs which is also responsible for the enhanced reactivity. Notwithstanding in the present kinetic runs, the substitution reactions were followed at a constant pH of 4.0 to avoid complication caused by adding an additional parameter of [H⁺] to the rate equation. At pH 4.0 the complex (I) exists in the diaqua form.

2.9 Effect of temperature on reaction rate

The reaction was studied at five different temperatures for different ligand concentrations and the anation rate constants for both A→B (k₁) and B→C (k₂) steps are given in Table 2.2. The activation parameters calculated from Eyring plots (Figures 2.9 and 2.10) are given in Table 2.3 and compared with those for analogous systems involving the substitution in square planar platinum(II) complexes.

![Figure 2.8](image_url)

**Figure 2.8:** Plots of 1 / \( k_{1\text{ (obs)}} \) (s) versus 1 / [DL-penicillamine] at different temperatures; A = 35 °, B = 40 °, C = 45 °, D = 50 ° and E = 55 °; pH = 4.0 and ionic strength = 0.1mol dm⁻³ NaClO₄.
**Table 2.3:** Activation parameters for analogous systems

<table>
<thead>
<tr>
<th>Systems</th>
<th>(\Delta H_1^\circ) (kJmol(^{-1}))</th>
<th>(\Delta S_1^\circ) (JK(^{-1})mol(^{-1}))</th>
<th>(\Delta H_2^\circ) (kJmol(^{-1}))</th>
<th>(\Delta S_2^\circ) (JK(^{-1})mol(^{-1}))</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis-[Pt(en)(H(_2)O)(_2)](^{2+})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>/DL-Methionine</td>
<td>15.6 ± 0.9</td>
<td>-230 ± 3</td>
<td>19.4 ± 1.2</td>
<td>-225.5 ± 4.0</td>
<td>[28]</td>
</tr>
<tr>
<td>/Thiourea</td>
<td>61.9 ± 1.7</td>
<td>-71 ± 6</td>
<td>26.7 ± 0.8</td>
<td>-186.8 ± 2.7</td>
<td>[29]</td>
</tr>
<tr>
<td>/Thiosemicarbazide</td>
<td>35.6 ± 0.8</td>
<td>-166 ± 3</td>
<td>44.5 ± 1.3</td>
<td>-182 ± 4</td>
<td>[30]</td>
</tr>
<tr>
<td>Cis-[Pt(cis-dach)(H(_2)O)(_2)](^{2+})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>/Glutathione</td>
<td>32.9 ± 1.3</td>
<td>-187.2 ± 4.2</td>
<td>30.5 ± 0.1</td>
<td>-223.1 ± 0.3</td>
<td>[31]</td>
</tr>
<tr>
<td>/Diethylidithiocarbamate</td>
<td>66.8 ± 3.7</td>
<td>-81 ± 12</td>
<td>95.1 ± 2.8</td>
<td>-34.4 ± 9.1</td>
<td>[32]</td>
</tr>
<tr>
<td>/DL-Penicillamine</td>
<td>36.1 ± 4.1</td>
<td>-175 ± 12</td>
<td>44.4 ± 1.1</td>
<td>-189 ± 3</td>
<td>[**]</td>
</tr>
</tbody>
</table>

**This work**

### 2.10 Mechanism and conclusion

DL-penicillamine exists as zwitterions at pH 4.0 (Scheme-1). The sulfur end of DL-penicillamine is a soft donor and has a large affinity for the soft Pt(II) centre. Thus in the first step, a rapid equilibrium is established, resulting an outer sphere association complex between complex (I) and DL-penicillamine. The two cis- position of platinum(II) ion are blocked by nitrogen ligands and in view of the preference for square planar configuration in its complex, it is unlikely that DL-penicillamine behave like a tridentate ligand in this complex formation. Job’s method of continuous variation indicate 1:1 molar ratio and the IR spectrum of the solid product suggests that DL-penicillamine is a bidentate ligand with carboxyl group free. Finally ESI-mass spectrum provides a qualitative picture of the composition i.e. the ligational sites are the sulfur and nitrogen ends of DL-penicillamine. Thus the mechanism of substitution of aqua ligands in cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) ion can be explained in terms of rapid outer sphere association complex formation, followed by two consecutive steps; the first is dependent on ligand concentration and the second is a chelation i.e. ring closure step, which is slower than the first step and independent of ligand concentration (Figure 2.11).
The affinity for nitrogen atom of the amino group provides the driving force for the ring formation. The activation parameters \( \Delta H^1 = 36.1 \pm 4.1 \text{ kJmol}^{-1}, \Delta S^1 = -175 \pm 12 \text{ JK}^{-1} \text{ mol}^{-1} \) for the first step and the second step \( \Delta H^2 = 44.4 \pm 1.1 \text{ kJmol}^{-1}, \Delta S^2 = -189 \pm 3 \text{ JK}^{-1} \text{ mol}^{-1} \) suggest an associative substitution. The enthalpy of activation \( \Delta H^1 \) and \( \Delta H^2 \) values and negative \( \Delta S^1 \) and \( \Delta S^2 \) values implies a good degree of ligand participation in the transition state. The positive enthalpy change for breaking the M-OH$_2$ bond is partially compensated by the formation of M-L bond in the transition state. The participation of DL-penicillamine in the transition state results in a more compact state and negative \( \Delta S^\prime \) is observed. Further \( \Delta S^2 \) is more negative than \( \Delta S^1 \), which suggest that compactness has already been achieved in B and the transformation of B to C is only the replacement of another aqua ligand through chelation. Aqua ligand substitution on cis-[Pt(cis-dach)-(H$_2$O)$_2$]$^{2+}$ complex with different sulphur donor ligands gives different enthalpy of activation \( (\Delta H^2) \) and entropy of activation \( (\Delta S^2) \) depending on the ligand. For the system cis-[Pt(cis-dach)(H$_2$O)$_2$]$^{2+}$ the enthalpy of activation \( (\Delta H^1) \) values are 32.9, 66.8 and 36.1 kJmol$^{-1}$ for glutathione, Et$_2$DTC$^-$ (diethyldithiocarbamate) and DL-penicillamine respectively (Table 2.3). In case of the Et$_2$DTC$^-$ highest enthalpy of activation is observed, which may be attributed to its inability to form hydrogen bond during substitution (outer sphere association) with leaving aqua ligand, whereas as glutathione and DL-penicillamine before deprotonation of –SH group are able to form hydrogen bond which finally leads to Pt-S bond. DL-penicillamine has higher \( \Delta H^1 \) than glutathione as glutathione have higher pK$_a$ for –S-H assisting glutathione to form better outer sphere association complex compared to DL-penicillamine. Similarly the enthalpies of activation \( (\Delta H^2) \) values are 30.5, 95.1 and 44.4 kJmol$^{-1}$ for ligands glutathione, Et$_2$DTC and DL-penicillamine respectively (Table 2.3).

Due to its great medical, biological and biochemical importance platinum chemistry is attracting considerable interest. Kinetic and mechanistic aspects of the reaction of DL-penicillamine with the title complex in aqueous medium extend the study of interaction platinum complexes with other biomolecules, providing new insights into this active research area.
**Figure 2.9:** Eyring plot for $k_1$

**Figure 2.10:** Eyring plot for $k_2$
Figure 2.11: Plausible mechanism for the substitution of the aqua ligand from \( \text{cis-}[\text{Pt(cis-dach)}(\text{H}_2\text{O})_2]^{2+} \) by DL-Penicillamine.
References


Chapter 3

Mechanistic aspects of ligand substitution on cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) by Glycyl-L-Leucine
3.1 Introduction

Nucleophilic substitution reactions on square planar cis-(N,N)-chelated platinum(II) complexes are of interest in the fields of chemical, catalytic and biomedical applications [1-7]. Cisplatin [cis-diamminedichloroplatinum(II)] is well known for its chemotherapeutic activity [8-11], but the drug has considerable adverse side effects [12-13]. Consequently, there is much interest in obtaining drugs that have lower toxicity and more favorable therapeutic indices [14]. Studies of the mechanism of action of platinum antitumor drugs have revealed that cis-diamine complexes of platinum(II) (non-chelated or chelated), containing at least one N-H bond on each nitrogen and two labile ligands, may serve as model anticancer compounds [15-16]. The complex cis-[Pt(cis-dach)Cl2] (dach = 1,2-diaminocyclohexane) fulfils these basic structural requirements and is also an anticancer agent, capable of circumventing cisplatin resistance and also showing reduced nephrotoxicity [17]. Due to the toxic characteristics of chloro complexes, aqua derivatives are now widely used, as the water ligand is a better leaving group than the chloro ligand in Pt(II) complexes and therefore one would expect the aqua species to react much faster in cells than the dichloro species.

DNA is the main target of these drugs in tumor cells [18]. However, there are many other biomolecules that can also potentially react with these Pt(II) complexes, such as small molecules, proteins and enzymes [19]. Examination of the modes of action of metal based antitumor drugs therefore requires the study of its interactions with a range of possible biological targets including amino acids, hormones, peptides and proteins. The kinetics and mechanism of the reactions of cis-(N,N)- chelated platinum(II) ions with proteins and their amino acid fragments are now a subject of interest [20-22]. The study of model species such as simple dipeptides can assist in the interpretation of more complex systems. This study has been undertaken in order to examine the reactivity of title complex towards dipeptides. In this chapter, the detailed kinetic and mechanistic studies of aqua ligand substitution from cis-[Pt(cis-dach)(OH2)2]2+ with the glycyl-L-leucine (Glyleu) a dipeptide in aqueous medium have been described.
3.2 Experimental

The reactant complex was prepared and characterized as described in previous chapter (Section 2.2). The pH of the solution was maintained at 4.0, so that perchlorate salt exists as diaqua species. The product of the reaction between complex (I) and Glyleu was prepared by mixing the reagents at pH 4.0 in different molar ratios, namely, 1:1, 1:2, 1:3, 1:4 and 1:5 and equilibrating the mixture at 60 °C for 48 h. The absorption spectra of the resulting solutions were recorded and all were found to exhibit almost identical absorbances at 227 nm. The spectral difference between the product complex and the substrate complex is shown in Figure 3.1.

![Figure 3.1: Spectral difference between complex (I) and the Glyleu substituted product; (1) [complex (I)] = 2.0 × 10⁻⁴ mol dm⁻³; (2) [complex (I)] = 2.0 × 10⁻⁴ mol dm⁻³; [Glyleu] = 6.0 × 10⁻³ mol dm⁻³; cell used: 1 cm quartz. The composition of the product in the solution was determined by Job’s method of continuous variation (Figure 3.2). The metal: ligand ratio was found to be 1:1.](image-url)
Figure 3.2: Job’s plot for the reaction between complex (I) and glycyl-L-leucine at pH = 4.0 and ionic strength = 0.1mol dm$^{-3}$ NaClO$_4$.

To further characterize the product, cis-[Pt(cis-dach)(OH$_2$)]$^{2+}$ and Glyleu were mixed in 1:1 molar ratio at pH 4.0 and a pale yellow solid was isolated. The IR spectrum of the product in the KBr disc showed strong bands in the region 3237 - 2957 cm$^{-1}$ together with medium bands at 1625, 532 and 420 cm$^{-1}$. The asymmetric COO$^-$ stretching frequency of the amino acids occurs at 1580-1660 cm$^{-1}$ when the group is coordinated to metals, whereas a non coordinated COO$^-$ group has the $\nu_{\text{asym}}$(COO$^-$) stretch at lower frequency [23]. The band at 1625 cm$^{-1}$ is therefore assigned to the $\nu_{\text{asym}}$(COO$^-$) of the metal bound carboxyl group. The band at 3237 - 3068 cm$^{-1}$ is assigned to an NH stretching frequency. The absence of a strong band at $\sim$ 3400 cm$^{-1}$ indicates the absence of free –OH, as observed in the free ligand. The bands at 532 and 420 cm$^{-1}$ are assigned to $\nu$(Pt-N) and $\nu$(Pt-O) respectively [24]. An intense band from the $\nu$(C-O)$_{\text{amide}}$ at 1691 cm$^{-1}$ in the non-coordinated Glyleu undergoes a bathochromic $\sim$ 37 cm$^{-1}$ shift in the IR spectrum upon complexation. This is probably due to the involvement of the deprotonated peptide nitrogen in bonding with Pt(II) [25]. The spectrum suggests that the final product is an (N, O) coordinated chelate in which Glyleu behaves as a bidentate ligand. The aqueous solution of cis-[Pt(cis-dach)(OH$_2$)$_2$]$^{2+}$ and Glyleu were mixed in a 1:1 molar ratio and the mixture was thermo stated at 60 $^\circ$C for 48 hours and used for ESI-MS measurement. The ESI mass spectrum of the resulting solution is shown in Figure 3.5. It is clear from this spectrum that the ion at m/z 496.2683 has become the precursor ion species in the mixture solution and this is tentatively attributed to [Glyleu + Pt(II) + dach]$^+$. 
These observations are consistent with other studies [26, 27] where (N, N)-platinum complexes bind to amino acids, substituted amino acids and dipeptides through O and N. The molar conductance measurement also provides evidence in favor of the interaction between cis-[Pt(cis-dach)(OH₂)]²⁺ and Glyleu. During chelation, one proton is released from the –CONH– (peptide linkage) group of Glyleu which is supported by an increase in conductance with the progress of the reaction.

3.3 Physical measurements
Spectra were recorded with a Shimadzu UV-Vis spectrophotometer (UV-2101 PC). IR Spectra (KBr disc, 4000 – 400 cm⁻¹) were measured with a Perkin-Elmer FTIR model RX1 Infrared spectrophotometer. ESI-mass spectrum was recorded using a micromass Q-Tof micro™ mass spectrometer in +ve ion mode. Conductance measurements were carried out with a Systronics conductivity meter model 308 where the cell constant was calibrated with 0.01 M KCl solution and water used as solvent. The pHs of the solutions were adjusted by adding NaOH/HClO₄ and the measurements were carried out with the help of a Sartorius Digital pH meter (model PB11) with an accuracy of ± 0.01 units.

**Figure 3.5:** ESI- mass spectrum of the product for complex (I) and the Glyleu substituted product at pH 4.0 in aqueous medium and at ionic strength 0.1 mol dm⁻³ NaClO₄.
Doubly distilled water was used to prepare all the solutions. All other chemicals used were of AR grade. The reactions were carried out at constant ionic strength (0.1 mol dm$^{-3}$ NaClO$_4$).

### 3.4 Kinetic measurements

Kinetic measurements were carried out on a Shimadzu spectrophotometer (UV-2101 PC) equipped with a Shimadzu TB 85 thermo bath (accuracy ± 0.1 °C). The absorption due to Glyleu was subtracted by using 1:1 (molar ratio) Glyleu:water mixture in the reference cell. The absorbance at 227 nm was monitored to follow the progress of the reaction. Conventional mixing technique was followed and pseudo first order conditions with respect to metal concentration were maintained throughout the course of the reactions. Plots of ln ($A_\infty - A_t$) (where $A_t$ and $A_\infty$ are absorbance at time (t) and after completion of reaction) against time (t) (Figure 3.3) were found to be nonlinear; being curved at the initial stage and subsequently of constant slope, indicating that the reaction proceeds via two consecutive steps. From the limiting linear portion of the curves, values of $k_2(\text{obs})$ were obtained. The $k_1(\text{obs})$ values were obtained from plots of ln$\Delta$ versus time (t) where time (t) is small (Figure 3.4). Origin software was used for computational analysis. Rate data, represented as an average of duplicate runs, were reproducible to within ± 4%.

![Figure 3.3: A typical kinetic plot of ln($A_\infty - A_t$) versus time. [complex ($I$)] = 2.0 × 10$^{-4}$ mol dm$^{-3}$; [Glyleu] = 8 × 10$^{-3}$ mol dm$^{-3}$; Temperature = 60 °C.](image-url)
Figure 3.4: A typical kinetic plot of lnΔ versus time. [complex (I)] = 2 × 10^{-4} \text{ mol dm}^{-3}; [Glyleu] = 8 × 10^{-3} \text{ mol dm}^{-3}; Temperature = 60 °C.

3.5 Results and discussion

The dipeptide glycyl-L-leucine contains three separate functional groups; a terminal amino group, terminal carboxylate group and an amide group. The two dissociation constants pK_1 (-COOH) and pK_2 (-NH_3^+) of Glyleu [28] are 3.18 and 8.14 at 25 °C respectively. So at pH 4.0 the major species involved in the kinetic process is the zwitterionic form of Glyleu. The pK_1 and pK_2 (6.25 and 7.80) for cis-(diaqua(cis-1,2-diaminocyclohexane)platinum(II) have been evaluated by Irving–Rossotti titration technique [29]. It can be assumed that at pH 4.0 the reactant exists as the diaqua ion. At constant temperature and pH 4.0 and fixed concentration of complex (I), the ln (A_{\infty} - A_t) versus time plots for different Glyleu concentrations were curved at the initial stage and subsequently of constant slope. This indicates that the reaction involves two-step consecutive processes; in the first step one aqua ligand is replaced from cis-[Pt(cis-dach)(OH_2)_2]^{2+} by Glyleu. The second step is slower, where another aqua ligand is substituted with chelate ring closure. The rate constant for such a process can be evaluated by assuming the following scheme:

\[ A \xrightarrow{k_1} B \xrightarrow{k_2} C \]

Where A is the diaqua species (I); B is the single substituted intermediate and C is the final product (2), [Pt(cis-dach)(Glyleu)]^+. Formation of C from B is predominant after some time has elapsed.
3.6 Calculation of $k_1$ for $A \rightarrow B$ step

The rate constant $k_{1\text{obs}}$ for the $A \rightarrow B$ step was evaluated by the method of Weyh and Hamm [30] as discussed in chapter 2 (Section 2.6). The value of $k_{1\text{obs}}$ was derived from the slope of $\ln \Delta$ versus $t$ (Correlation Coefficient 0.9986), for small $t$ (Figure 3.4).

A similar procedure was applied for each Glyleu in the concentration in the range of 0.002 – 0.010 mol dm$^{-3}$ at constant complex (I) concentration of 0.0002 mol dm$^{-3}$ at pH 4.0 and at 40, 45, 50, 55 and 60 °C respectively. The $k_{1\text{obs}}$ values thus obtained are linearly dependent on the studied concentration range. The $k_{1\text{obs}}$ values for different Glyleu concentrations at different temperatures are given in Table 3.1.

Table 3.1: $10^3 k_{1\text{obs}}$ (s$^{-1}$) values for different concentrations of Glyleu at different temperatures. $[\text{complex (I)}] = 2 \times 10^{-4}$ mol dm$^{-3}$, pH = 4.0, $\mu = 0.1$ mol dm$^{-3}$ NaClO$_4$.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>$10^3$ [Glyleu] (mol dm$^{-3}$)</th>
<th>2.00</th>
<th>4.00</th>
<th>6.00</th>
<th>8.00</th>
<th>10.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.30</td>
<td>0.61</td>
<td>0.90</td>
<td>1.20</td>
<td>1.51</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>0.45</td>
<td>0.90</td>
<td>1.36</td>
<td>1.81</td>
<td>2.27</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.59</td>
<td>1.17</td>
<td>1.78</td>
<td>2.38</td>
<td>2.97</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>0.77</td>
<td>1.54</td>
<td>2.32</td>
<td>3.08</td>
<td>3.87</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1.08</td>
<td>2.17</td>
<td>3.24</td>
<td>4.33</td>
<td>5.42</td>
<td></td>
</tr>
</tbody>
</table>

The following scheme 3.1 is proposed for the reaction:

\[
\text{cis-[Pt(cis-dach)(H_2O)\text{2}^+ + GlyleuH} \xrightarrow{k_1, \text{slow} \text{ anation}} \text{cis-[Pt(cis-dach)(H_2O)(GlyleuH)]\text{2}^+ + H_2O}
\]

\[
\text{cis-[Pt(cis-dach)(H_2O)(GlyleuH)]\text{2}^+} \xrightarrow{k_2 \text{ chelation}} \text{cis-[Pt(cis-dach)(Glyleu)]}^+ + H_3O^+
\]

Scheme 3.1

Where GlyleuH is the zwitterionic form of glycyl-L-leucine. Based on the above scheme, a rate expression (3.5) can be derived for the $A \rightarrow B$ step:

\[
\frac{dB}{dt} = k_1[\text{Pt(dach)(H}_2\text{O)}\text{2}^+\text{total}[\text{Glyleu}]
\]

(3.4)

Where $[\text{Pt(dach)(H}_2\text{O)}\text{2}^+\text{total}$ is the concentration of the unreacted complex. Hence it can be written:
\[ k_{1(\text{obs})} = k_1 [\text{Glyleu}] \]  

where \( k_1 \) is the second order rate constant for the first aqua ligand substitution. A plot of \( k_{1(\text{obs})} \) (s\(^{-1}\)) versus [Glyleu] should be linear, passing through the origin. Absence of any intercept indicates the completion of the reaction and absence of reversibility. This was found to be so at all temperatures studied (Figure 3.6). No evidence in favour of outersphere association complex formation was found in the studied concentration range. The second-order rate constants \( (k_1) \) calculated from the slopes of the plot of \( k_{1(\text{obs})} \) (s\(^{-1}\)) versus [Glyleu] (mol dm\(^{-3}\)) at different temperatures are given in Table 3.2.

![Figure 3.6: Plots of \( k_{1(\text{obs})} \) (s\(^{-1}\)) versus [Glyleu] at different temperatures. A = 40, B = 45, C = 50, D = 55 and E = 60 °C](image)

3.7 Calculation of \( k_2 \) for B \( \rightarrow \) C step

The B \( \rightarrow \) C step is assigned to ring closure in which the amide group of the peptide linkage of the dipeptide binds the metal centre through N. Due to the steric hindrance, this chelation step is slower and independent of ligand concentration. At each temperature, the \( k_2 \) values were calculated from the limiting linear portions (when t is large) of the \( \ln(A_\infty - A_t) \) versus t curves and are collected in Table 3.2. Unlike \( k_1 \), \( k_2 \) was found to be independent of ligand concentration at each of the temperatures studied.
Table 3.2: $k_1$ and $k_2$ values for the substitution reaction

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$k_1$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</th>
<th>$10^5 k_2$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.15</td>
<td>2.01</td>
</tr>
<tr>
<td>45</td>
<td>0.23</td>
<td>2.63</td>
</tr>
<tr>
<td>50</td>
<td>0.30</td>
<td>3.68</td>
</tr>
<tr>
<td>55</td>
<td>0.39</td>
<td>5.15</td>
</tr>
<tr>
<td>60</td>
<td>0.54</td>
<td>7.22</td>
</tr>
</tbody>
</table>

3.8 Effect of pH on reaction rate

The reaction was studied at four different pH values. The $k_{obs}$ values were found to increase with increase in pH. At fixed concentrations of 0.0002 mol dm$^{-3}$ of [complex(I)], 0.008 mol dm$^{-3}$ [Glyleu] and 0.1 mol dm$^{-3}$ ionic strength the $10^3 k_{1(obs)}$ values at 55 °C were 1.32, 2.28, 3.08 and 3.71 s$^{-1}$ and $10^5 k_{2(obs)}$ values were 2.71, 3.66, 5.15 and 7.38 s$^{-1}$ at pH 2.8, 3.4, 4.0 and 4.6 respectively. The increase in rate may be explained based on the acid dissociation equilibria of the Glyleu and the complex. The enhancement in rate may be explained based on two acid dissociation equilibria of the ligand and the complex. In the studied pH range with increase in pH, the percentage of hydroxoaqua complex is increased and the reactivity of hydroxoaqua complex is usually higher than that of diaqua complex by the well-known labilising effect of the coordinated hydroxide ion via its p-bonding ability and strong electromeric effect. At the same time, with increase in pH deprotonation of the ligand occurs which is also responsible for the enhanced reactivity. In subsequent kinetic runs, the substitution reactions were followed at a constant pH of 4.0 to avoid complications from an additional parameter of [H$^+$] to the rate equation.

3.9 Effect of temperature on reaction rate

To study the effect of temperature, the reaction was studied at five different temperatures for different Glyleu concentrations and the anation rate constants for both $k_1$ and $k_2$ steps are given in Table 3.2. The activation parameters calculated from Eyring plots ($R^2$ for $k_1$ is 0.9914 and $R^2$ for $k_2$ is 0.9971) are given in Table 3.3 and compared with those for analogous systems involving the substitution in square planar platinum(II) complexes.
Table 3.3: Activation parameters for analogous systems

<table>
<thead>
<tr>
<th>Systems</th>
<th>$\Delta H_1^z$ (kJmol$^{-1}$)</th>
<th>$\Delta S_1^z$ (JK$^{-1}$mol$^{-1}$)</th>
<th>$\Delta H_2^z$ (kJmol$^{-1}$)</th>
<th>$\Delta S_2^z$ (JK$^{-1}$mol$^{-1}$)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis-[Pt(en)$_2$(H$_2$O)$_2$]$^{2+}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>/L-Asparagine</td>
<td>$43.59 \pm 0.96$</td>
<td>$-116.98 \pm 2.9$</td>
<td>$33.78 \pm 0.51$</td>
<td>$-221.43 \pm 1.57$</td>
<td>[31]</td>
</tr>
<tr>
<td>/Thiourea</td>
<td>$61.9 \pm 1.7$</td>
<td>$-71 \pm 6$</td>
<td>$26.7 \pm 0.8$</td>
<td>$-186.8 \pm 2.7$</td>
<td>[32]</td>
</tr>
<tr>
<td>/Thiosemicarbazide</td>
<td>$35.6 \pm 0.8$</td>
<td>$-166 \pm 3$</td>
<td>$44.5 \pm 1.3$</td>
<td>$-182 \pm 4$</td>
<td>[33]</td>
</tr>
<tr>
<td>Cis-[Pt(cis-dach)(H$_2$O)$_2$]$^{2+}$</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>/Glutathione</td>
<td>$32.9 \pm 1.3$</td>
<td>$-187.2 \pm 4.2$</td>
<td>$30.5 \pm 0.1$</td>
<td>$-223.1 \pm 0.3$</td>
<td>[34]</td>
</tr>
<tr>
<td>/DL-Penicillamine</td>
<td>$36.1 \pm 4.1$</td>
<td>$-175 \pm 12$</td>
<td>$44.4 \pm 1.1$</td>
<td>$-189 \pm 3$</td>
<td>[35]</td>
</tr>
<tr>
<td>/Glycyl-L-Leucine</td>
<td>$51.9 \pm 2.8$</td>
<td>$-152 \pm 8$</td>
<td>$54.4 \pm 1.7$</td>
<td>$-162 \pm 5$</td>
<td>[**]</td>
</tr>
</tbody>
</table>

** This work

3.10 Mechanism and conclusion:

The dipeptide glycyl-L-leucine exists as a zwitterion at pH 4.0. The present investigation on substitution of aqua ligands from cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) by Glyleu shows that the dipeptide interacts in an associative mode of activation in the transition state. The second step is the chelate ring closure step, which is slower than the first step and independent of Glyleu concentration (Figure 3.7). The two cis positions of platinum(II) are blocked by the NH$_2$ groups of the dach ligand and in view of the preference of square planar configuration in platinum(II) complexes it is unlikely that Glyleu behaves as a tetradentate ligand in the product complex. Job’s method of continuous variation indicates a 1:1 M ratio, and the IR spectrum of the product suggests that Glyleu behaves as a bidentate ligand for which Pt(II) is able to promote amide deprotonation [36]. The activation parameters ($\Delta H_1^z = 51.9 \pm 2.8$ kJmol$^{-1}$, $\Delta S_1^z = -152 \pm 8$ JK$^{-1}$ mol$^{-1}$) for the first and second steps ($\Delta H_2^z = 54.4 \pm 1.7$ kJmol$^{-1}$, $\Delta S_2^z = -162 \pm 5$ JK$^{-1}$ mol$^{-1}$) suggest an associative mode of activation for the substitution processes. The $\Delta H_1^z$ and $\Delta H_2^z$ values and negative $\Delta S_1^z$ and $\Delta S_2^z$ values imply a good degree of ligand participation in the transition state. The aqua ligand substitution reactions of cis-[Pt(cis-dach)(H$_2$O)$_2$]$^{2+}$ with different donor ligands give different enthalpies and entropies of activation depending on the character of the
incoming ligands (Table 3.3). The higher enthalpy of activation of Glyleu can be explained by its weak ability to form hydrogen bonds during substitution (outer sphere association) with the leaving aqua ligand, whereas glutathione and DL-penicillamine are able to form such hydrogen bonds by the \(-\text{SH}\) group.

![Plausible mechanism for the substitution of the aqua ligand from \(\text{cis-}[\text{Pt(cis-dach)}(\text{H}_2\text{O})_2]^{2+}\) by glycyll-L-leucine; \(R = -\text{CH}_2\text{CHMe}_2\).]
References


Chapter 4

Kinetics and mechanism of the interaction of adenosine with cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) perchlorate in aqueous medium
**4.1 Introduction**

In continuation of the earlier studies, it is observed that binding of platinum(II) to purine nucleosides and related compounds are of interest and the earlier research on the platinum complexes with them had attracted much attention due to their potential applications as anticancer medications [1-3]. Much emphasis has been paid especially to the coordination properties of the base moieties because certain anticarcinogenic platinum(II) compounds are believe to interact directly with purine base in DNA malignant cells[4-7]. This chapter describes the detailed kinetic and mechanistic studies of aqua ligand substitution from cis-[Pt(cis-dach)(OH$_2$)$_2$]$^{2+}$ by adenosine, a ubiquitous nucleoside of adenine and dephosphorylation product of adenine nucleotide, ATP. Therefore, this study will throw some light in in-vitro interaction of cis-[Pt(cis-dach)(OH$_2$)$_2$]$^{2+}$ with adenosine and measures the effectiveness of this antitumor drug.

**4.2 Experimental**

The reactant complex was prepared and characterized as described in previous chapter (Section 2.2). The pH of the solution was maintained at pH (4.0), so that perchlorate salt exists as diaqua species. The product of the reaction between complex (I) and adenosine was prepared by mixing the reactants at pH 4.0 in different molar ratio: viz 1:1, 1:2, 1:3, 1:4 and 1:5 and thermostating the mixture at 60 °C for 48 hours. The absorption spectra of the resulting solutions were recorded and all were found to exhibit almost identical absorbances at 287 nm wavelength. The difference in spectra between the product complex and the substrate complex is shown in Figure 4.1.
Figure 4.1: Spectral difference between complex (I) and the adenosine substituted product; (1) [complex (I)] = $2.0 \times 10^{-4}$ mol dm$^{-3}$; (2) [complex (I)] = $2.0 \times 10^{-4}$ mol dm$^{-3}$; [Adenosine] = $6.0 \times 10^{-3}$ mol dm$^{-3}$. 1 cm, quartz cell.

The composition of the product in the reaction mixture was determined by Job’s method of continuous variation (Figure 4.2). The metal: ligand ratio was found to be 1:1.

Figure 4.2: Job’s plot for the reaction between complex (I) and adenosine at pH = 4.0 and ionic strength = 0.1mol dm$^{-3}$ NaClO$_4$. Total molar concentration i.e., [L] + [M] = $1 \times 10^{-4}$ mol dm$^{-3}$ and 287 nm wavelength used for the series.
Cis-[Pt\((\text{cis-dach})(\text{OH}_2)\)]^{2+} and adenosine were mixed in 1:1 molar ratio at pH 4.0 and a yellowish-brown solid was isolated. The IR spectrum of it in the KBr disc shows strong bands at 3441, 1654 and 1638 cm\(^{-1}\) together with medium bands at 3200-3100 cm\(^{-1}\) and 538 cm\(^{-1}\). The presence of strong stretching band at \(\sim 3441\) cm\(^{-1}\) indicates the presence of free –OH groups or the product is hydrated. The band at 3299-2928 cm\(^{-1}\) is assigned to overlapping of \(\nu_{(\text{asym})}\) and \(\nu_{(\text{sym})}\) motion of the –NH\(_2\) group coordinated to platinum(II) in the product complex. The band at 538 cm\(^{-1}\) is also assigned as \(\nu(\text{Pt-N})\) bond formation \([8-10]\). The band at 1600 cm\(^{-1}\) in the spectrum of adenosine mainly due to skeletal vibration, gained intensity and shifted to 1638 cm\(^{-1}\). This shift is due to inductive effect of the metal ion electrophile bound to N\(_7\), which causes less electron delocalization, and finally less contribution to the skeletal vibration of the ring system \([11]\). The intense band around 1680 cm\(^{-1}\) of adenosine was due to bending mode of C\(_6\)-NH\(_2\) group and this was found to be greatly reduce and shifted to 1654 cm\(^{-1}\). It is attributed by the C\(_6\)-NH\(_2\) coordination in the metal complex \([12]\). Shifting and sharpening of the NH\(_2\) stretching vibration at 3400 cm\(^{-1}\) to a higher frequency also indicates metalation of C\(_6\)-NH\(_2\). From the individual assignments of different bands it can be presumed that the final product is a (N, N) coordinated chelate and adenosine behaves like a bidentate ligand at the experimental pH. The aqueous solutions of cis -[Pt\((\text{cis-dach})(\text{OH}_2)\)]^{2+} and adenosine were mixed in a 1:1 molar ratio and the mixture was thermostated at 60 °C for 48 hours and used for ESI-MS measurement. The ESI mass spectra of the resulting solution are shown in Figure 4.3.
Figure 4.3: ESI-mass spectrum of the product i.e. adenosine substituted cis-[Pt(cis-
dach)(OH$_2$)$_2$]$^{2+}$ complex.
It is clear from this spectrum that the ion at m/z 675.7031 (moderate peak) has become the precursor ion species in the mixture solution and this is tentatively attributed to $[\text{adenosine} + \text{Pt(II)} + \text{dach} + \text{ClO}_4^-]^+$ (Figure 4.4). The relative abundance of the isotopic peaks matched the expected values very well, i.e., m/z 674.6974 $\sim$ 32%, m/z 675.7031 $\sim$ 34%, m/z 676.7093 $\sim$ 25%, m/z 678.6962 $\sim$ 7%. Therefore, the m/z values and isotope distribution pattern assigned that the product is adenosine substituted complex (I).

![Figure 4.4](image)

**Figure 4.4**: Plausible structure of the precursor ion (ion-pair) matched with that m/z value obtained from the ESI-mass spectrum.

Pt(II) is likely to be coordinated to both the exocyclic NH$_2$(C$_6$) group and N(7) nitrogen of the purine ring considering the strong coordination ability of these functional groups [13-15]. Thus the structure proposed here for product ion species, deduced from ESI-mass spectra, is generally consistent with those derived from other experimental methods. Therefore this study provides more insights into the most likely binding mechanism of the platinum(II) complex with DNA.

### 4.3 Physical measurements

Spectra were recorded with a Shimadzu UV-Vis spectrophotometer (UV-2101 PC). IR Spectra (KBr disc, 4000 – 400 cm$^{-1}$) were measured with a Perkin-Elmer FTIR model RX1 Infrared spectrophotometer. ESI-mass spectrum was recorded using a micromass Q-Tof micro™ mass spectrometer in +ve ion mode. Conductance measurements were carried out with a Systronics conductivity meter model 308 where the cell constant was calibrated with 0.01 M KCl solution and water used as solvent. The pHs of the solutions
were adjusted by adding NaOH/HClO₄ and the measurements were carried out with the help of a Sartorius Digital pH meter (model PB11) with an accuracy of ± 0.01 units. Doubly distilled water was used to prepare all the solutions. All other chemicals used were of AR grade. The reactions were carried out at constant ionic strength (0.1 mol dm⁻³ NaClO₄).

4.4 Kinetic measurements

Kinetic measurements were carried out on a Shimadzu spectrophotometer (UV-2101 PC) equipped with a Shimadzu TB 85 thermo bath (accuracy ± 0.1 °C). The absorption due to adenosine was subtracted by using 1:1 (molar ratio) ligand: water mixture in the reference cell. The absorbance at 287 nm was monitored to follow the progress of the reaction. Conventional mixing technique was followed and pseudo first order conditions with respect to metal ion concentration, were maintained throughout the course of the reaction. A typical plot of ln (A∞ - A₁) (where A₁ and A∞ are absorbances at time and after completion of the reaction) against time (t) (Figure 4.5) is found to be nonlinear; it is curved at initial stage and subsequently of constant slope indicating that the reaction proceeds via two consecutive steps. From the limiting linear portion of ln (A∞ - A₁) versus time (t) curve, k₂(obs) was obtained. The k₁(obs) values were obtained from the plot of lnΔ versus time (t) where time (t) is small (Figure 4.6). Origin software was used for computational works. Rate data, represented as an average of duplicate runs, are reproducible within ± 4%.

Figure 4.5: A typical kinetic plot of ln( A∞ - A₁ ) versus time (t). [complex (I)] = 2.0 × 10⁻⁴ mol dm⁻³; [adenosine] = 6 × 10⁻³ mol dm⁻³; Temperature = 60 °C.
Figure 4.6: A typical kinetic plot of lnΔ versus time (t). [complex \((I)\)] = 2 × 10^{-4} \text{ mol dm}^{-3}; [adenosine] = 6 × 10^{-3} \text{ mol dm}^{-3}; Temperature = 60 °C.

4.5 Results and discussion

The pK\(_1\) and pK\(_2\) values \[16\] of adenosine (LH) are 3.5 and 12.5 at 25 °C respectively, which refer to following dissociation processes:

\[ \text{LH}_2^+ \rightleftharpoons \text{LH} + \text{H}^+ , \quad \text{pK}_1 = 3.5 \]

\[ \text{LH} \rightleftharpoons \text{L}^- + \text{H}^+ , \quad \text{pK}_2 = 12.5 \]

Scheme 4.1

So at pH 4.0 the major species involved in the kinetic process is neutral form of adenosine. The pK\(_1\) and pK\(_2\) (6.25 and 7.80) for \textit{cis-}(diaqua\textit{cis-1,2-diaminocyclohexane})platinum(II) has been evaluated by Irving–Rossotti titration technique \[17\]. Hence it can be assumed that at pH 4.0 the reactant exists as the diaqua ion. The reaction involves a two-step consecutive process; the first step is dependent on ligand concentration whereas the second one is the independent of ligand concentration. In the first step one aqua ligand was replaced from \textit{cis -} [Pt\textit{cis-dach}(OH\textsubscript{2})\textsubscript{2}]\textsuperscript{2+} by adenosine and in the slower step, where another aqua ligand is substituted, which is a ring closure step. The rate constant for such a process can be evaluated by assuming the following scheme:

\[ \text{A} \xrightarrow{k_1} \text{B} \xrightarrow{k_2} \text{C} \]
Where $A$ is the diaqua species ($I$); $B$ is the single substituted intermediate with monodentate LH and $C$ is the final product ($2$) $[\text{cis-Pt(cis-dach)(LH)}]^+$. Formation of $C$ from $B$ is predominant after some time has elapsed.

### 4.6 Calculation of $k_1$ for $A \rightarrow B$ step

The rate constant $k_{1(\text{obs})}$ for the $A \rightarrow B$ step can be evaluated by the method of Weyh and Hamm [18] as discussed in chapter 2 (Section 2.6). $k_{1(\text{obs})}$ is derived from the slope of $\ln \Delta$ versus time plot (correlation coefficient 0.998), when time ($t$) is small (Figure 4.6). A similar procedure is applied for each ligand in the concentration range of 0.002 – 0.006 mol dm$^{-3}$ at constant complex ($I$) concentration of 0.0002 mol dm$^{-3}$ at pH 4.0 and at 40, 45, 50, 55 and 60 °C respectively. The $k_{1(\text{obs})}$ values thus obtained are linearly dependent on ligand concentration over the studied range. However, studies at further higher concentrations were restricted due to the solubility problem of adenosine molecule in aqueous medium. The $k_{1(\text{obs})}$ values for different ligand concentrations at different temperatures are given in Table 4.1. The ligand concentration dependence of $k_{1(\text{obs})}$ values can be explained by considering the following scheme 4.1.

\[
\begin{align*}
\text{A} & \xrightarrow{k_{1, \text{slow}}} \text{B} \\
\text{B} & \xrightarrow{k_2} \text{C}
\end{align*}
\]

**Scheme 4.1**

Where Aden is the neutral form of adenosine ligand. Based on the above scheme, a rate expression (4.5) can be derived for the $A \rightarrow B$ step:

\[
\frac{d[B]}{dt} = k_1[\text{cis-Pt(cis-dach)(H}_2\text{O)}_2^{2+}]_{\text{total}}[\text{adenosine}] 
\]

Where $[\text{cis-Pt(cis-dach)(H}_2\text{O)}_2^{2+}]_{\text{total}}$ is the concentration of the unreacted complex and [adenosine] is the concentration of adenosine. Hence it can be written:

\[
k_{1(\text{obs})} = k_1[\text{adenosine}] 
\]

where $k_1$ is the second order rate constant for first step of aqua ligand substitution. A plot of $k_{1(\text{obs})}$ (s$^{-1}$) versus [adenosine] should be linear, passing through origin. Absence of any intercept indicates the completion of the reaction and absence of reversibility. This was found to be so at all temperatures studied (Figure 4.7). No evidence in favour of
outersphere association complex formation was found as the study was restricted to higher concentration. The second-order rate constants ($k_1$) calculated from the slopes of $k_{1\text{(obs)}}$ (s$^{-1}$) versus [adenosine] (mol dm$^{-3}$) plots at different temperatures are given in Table 4.2.

**Table 4.1**: $10^3 k_{1\text{(obs)}}$ (s$^{-1}$) values for different concentrations of adenosine at different temperatures. [complex (I)] = 2 × 10$^{-4}$ mol dm$^{-3}$, pH = 4.0, $\mu$ = 0.1 mol dm$^{-3}$ NaClO$_4$.

<table>
<thead>
<tr>
<th>Temp. ($^\circ$C)</th>
<th>10$^3$ [adenosine] (mol dm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.00</td>
</tr>
<tr>
<td>40</td>
<td>0.41</td>
</tr>
<tr>
<td>45</td>
<td>0.55</td>
</tr>
<tr>
<td>50</td>
<td>0.73</td>
</tr>
<tr>
<td>55</td>
<td>0.95</td>
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<tr>
<td>60</td>
<td>1.21</td>
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</tbody>
</table>

**Figure 4.7**: Plots of $k_{1\text{(obs)}}$ (s$^{-1}$) versus [adenosine] at different temperatures and pH = 4.0. A = 40°C, B = 45°C, C = 50°C, D = 55°C and E = 60°C

**4.7 Calculation of $k_2$ for the B→C step**

The B →C step is the ring closure step and is independent of ligand concentration. At a particular temperature the slopes of $\ln(A_{\infty} - A_t)$ versus time(t) plots for different ligand
concentrations were found to be constant in the region where plots are linear (Figure 4.5). For different temperatures the $k_2$ values are obtained directly from the limiting slopes and are collected in Table 4.2. The experimental results show a similar curvature of $\ln(A_{\infty} - A_t)$ versus time($t$) plots at different temperatures for varying concentrations. The assumption of two consecutive steps for such a reaction and the computation of $k_1$ and $k_2$ values fit well with the experimental values.

**Table 4.2:** $k_1$ and $k_2$ values for the substitution reaction, $\{I\} = 2 \times 10^{-4}$ mol dm$^{-3}$, pH = 4.0, $\mu = 0.1$ mol dm$^{-3}$ NaClO$_4$.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$k_1$ (mol$^{-1}$dm$^3$s$^{-1}$)</th>
<th>$10^3 k_2$ (s$^{-1}$)</th>
</tr>
</thead>
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<tr>
<td>40</td>
<td>0.21</td>
<td>2.32</td>
</tr>
<tr>
<td>45</td>
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</tr>
<tr>
<td>50</td>
<td>0.37</td>
<td>4.29</td>
</tr>
<tr>
<td>55</td>
<td>0.48</td>
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</tr>
<tr>
<td>60</td>
<td>0.61</td>
<td>7.44</td>
</tr>
</tbody>
</table>

**4.9 Effect of temperature on reaction rate**

The reaction was studied at five different temperatures for different ligand concentration and the anation rate constants for both $A \rightarrow B$ ($k_1$) and $B \rightarrow C$ ($k_2$) steps are given in Table 4.2. The activation parameters calculated from Eyring plots (Figures 4.8 and 4.9) are given in Table 4.3 and compared with those for analogous systems involving the substitution in square planar platinum(II) complexes.

![Figure 4.8: Eyring plot for $k_1$](image-url)
4.9 Effect of pH on reaction rate

The reaction was studied at four different pH values. The $k_{\text{obs}}$ values are found to increase with increase in pH in the studied pH range. At a fixed 0.0002 mol dm$^{-3}$ [complex($I$)], 0.005 mol dm$^{-3}$ [adenosine] and 0.1 mol dm$^{-3}$ ionic strength (NaClO$_4$) the $10^3k_{1(\text{obs})}$ values at 50 °C in aqueous medium are 0.38, 0.90, 1.84 and 2.11 s$^{-1}$ and $10^5k_{2(\text{obs})}$ values are 1.91, 2.66, 4.29 and 6.88 s$^{-1}$ at pH 2.8, 3.4, 4.0 and 4.6 respectively. The pH dependence of both $k_{1(\text{obs})}$ and $k_{2(\text{obs})}$ (Figure 4.10) can be readily interpreted in terms of the changes with pH in fractional populations of species involved in the respective reactions. For step 1, the reaction with adenosine, the reactive species in the pH range investigated are likely to be the neutral adenosine species, denoted HAd, and [Pt(dach)(H$_2$O)$_2$]$^{2+}$. The fraction of free adenosine in the HAd form in this pH range, $f_{\text{HAd}}$, is given by $K_1/([H^+] + K_1)$, where $K_1$ is the first dissociation constant of H$_2$Ad$^+ = 3.5$, so the actual concentration of HAd is just $f_{\text{HAd}} \times [\text{Ad}]_{\text{tot}}$. So the rate = $k_{1(\text{obs})}[\text{Pt(dach)(H}_2\text{O)}]_2^{2+} = k_1[\text{HAd}][\text{Pt(dach)(H}_2\text{O)}]_2^{2+} = k_1f_{\text{HAd}}[\text{Ad}]_{\text{tot}}[\text{Pt(dach)(H}_2\text{O)}]_2^{2+}$. For a series of pH values at constant [Ad]$_{\text{tot}}$, a plot of $k_{1(\text{obs})}$ versus $f_{\text{HAd}}$ should be linear with slope = $k_1[\text{Ad}]_{\text{tot}}$ and intercept at the origin, for the pH data for runs at 50°C (Figure 4.11). The second-order rate constant is thus $2.334 \times 10^{-3}/0.005 = 0.47$ dm$^3$ mol$^{-1}$s$^{-1}$ For step 2, the acid-base equilibrium to consider is the dissociation of the coordinated water of [Pt(dach)(H$_2$O)(HAd)]$^{2+}$, the product of the first step in the overall reaction to form the chelated adenosine. Based on comparable [PtN$_3$(H$_2$O)]$^{2+}$ species, the pKa for the
coordinated water proton should be about 4.0-5.0. The fractional population of deprotonated species was calculated for the different pH values for several assumed pKa values and the $k_{2(\text{obs})}$ values were plotted versus these fractional populations for each pKa. The curves show pronounced negative curvature for pH less than about 4.3 and pronounced positive curvature for pH greater than 4.3. The linear plot of the data at pH 4.3 yields a slope $= 7.7 \times 10^{-5}$ sec$^{-1}$ and an intercept of $1.7 \times 10^{-5}$ sec$^{-1}$ (Figure 4.12). These could be interpreted as the rate constants for intra-molecular ring closure via $[\text{Pt(dach)}(\text{OH})(\text{HAd})]^+$ and $[\text{Pt(dach)}(\text{H}_2\text{O})(\text{HAd})]^2+$ respectively. In each case a protonated NH$_3^+$ of the unidentate coordinated adenosine must lose its proton in the process of coordinating to the Pt(II) center. The foregoing analysis provides a quantitative explanation for the distinctly different effect of pH on the two observed rate constants. The rate of the first step levels off after about pH 4, while the rate of the ring closure increases more rapidly after pH 4. The pKa$_1$ of protonated adenosine, 3.5, insures that the relative concentration of deprotonated adenosine levels off by pH 4.5. By contrast, the intramolecular second step is dependent on deprotonation of the coordinated water of the initial product. The estimated pKa of about 4.3 requires that the extent of deprotonation and the associated ring closure rate increases most rapidly from 4.0 - 4.6.

Figure 4.10: Effect of pH on $k_{(\text{obs})}$
Figure 4.11: Effect of pH on $k_{1\text{obs}}$ at 50 °C

Figure 4.12: The rate constant, $k_{2\text{obs}}$, *versus* fractional population for assumed pKa values of [Pt(dach)(H$_2$O)(HAd)]$^{2+}$.
Table 4.3: Activation parameters for analogous systems

<table>
<thead>
<tr>
<th>Systems</th>
<th>ΔH₁ ‡</th>
<th>ΔS₁ ‡</th>
<th>ΔH₂ ‡</th>
<th>ΔS₂ ‡</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis-[Pt(en)₂(H₂O)₂]²⁺ / L-Methionine</td>
<td>52 ± 2</td>
<td>-82 ± 7</td>
<td>52 ± 2</td>
<td>-92 ± 4</td>
<td>[19]</td>
</tr>
<tr>
<td>/Thiourea</td>
<td>61.9 ± 1.7</td>
<td>-71 ± 6</td>
<td>26.7 ± 0.8</td>
<td>-186.8 ± 2.7</td>
<td>[20]</td>
</tr>
<tr>
<td>/Thiosemicarbazide</td>
<td>35.6 ± 0.8</td>
<td>-166 ± 3</td>
<td>44.5 ± 1.3</td>
<td>-182 ± 4</td>
<td>[21]</td>
</tr>
<tr>
<td>Cis-[Pt(cis-dach)(H₂O)₂]²⁺ /L-Methionine</td>
<td>51 ± 2</td>
<td>-76 ± 5</td>
<td>54 ± 6</td>
<td>-137 ± 20</td>
<td>[19]</td>
</tr>
<tr>
<td>/DL-Penicillamine</td>
<td>36.1 ± 4.1</td>
<td>-175 ± 12</td>
<td>44.4 ± 1.1</td>
<td>-189 ± 3</td>
<td>[22]</td>
</tr>
<tr>
<td>/Glutathione</td>
<td>32.9 ± 1.3</td>
<td>-187.2 ± 4.2</td>
<td>30.5 ± 0.1</td>
<td>-223.1 ± 0.3</td>
<td>[23]</td>
</tr>
<tr>
<td>/Adenosine</td>
<td>43.1 ± 1.3</td>
<td>-177 ± 4</td>
<td>47.9 ± 1.8</td>
<td>-181 ± 6</td>
<td>[** ]</td>
</tr>
</tbody>
</table>

** This work

4.10 Mechanism and conclusions

The present investigation of substitution of aqua ligands in cis-diaqua(cis-1, 2-diaminocyclohexane)platinum(II) ion by adenosine shows that adenosine interacts in an associative mode of activation in the transition state. The second step is the chelation i.e. ring closure step, which is slower than the first step and independent of ligand concentration (Figure 4.13). The two cis positions of platinum(II) ion are blocked by -NH₂ group of dach and in view of preference for a square planar configuration in its complexes, it is likely that adenosine behaves as a bidentate ligand in the complex formation. The result of Job’s method of continuous variation, (1:1 M:L molar ratio) and the IR spectrum of the solid product suggests that adenosine behaves as a bidentate ligand. Finally, ESI-mass spectrum provides a qualitative picture of the composition of the product. The affinity for nitrogen atom of the amino group on adenosine provides the driving force for the ring formation. During the reaction adenosine forms stable linkage with complex (I) by N-7 position as platinum binding to the N-7 atom of adenosine is stabilized by intramolecular hydrogen bonding between a coordinated water ligand molecule and the exocyclic –NH₂ group [24].
The activation parameters for the first ($\Delta H_1^z = 43.1 \pm 1.3 \text{ kJmol}^{-1}$, $\Delta S_1^z = -177 \pm 4 \text{ JK}^{-1} \text{ mol}^{-1}$) and the second step ($\Delta H_2^z = 47.9 \pm 1.8 \text{ kJmol}^{-1}$, $\Delta S_2^z = -181 \pm 6 \text{ JK}^{-1} \text{ mol}^{-1}$) suggest an associative mode of activation for the substitution process. The enthalpy of activation ($\Delta H_1^z$ and $\Delta H_2^z$) values and negative entropy ($\Delta S_1^z$ and $\Delta S_2^z$) values imply a good degree of ligand participation in the transition state. The positive enthalpy change for breaking the M-OH$_2$ bond is partially compensated by the formation of M-L bond in the transition state. The participation of adenosine in the transition state results in a more compact state and negative $\Delta S^z$ value is obtained. Further, $\Delta S_2^z$ is more negative than $\Delta S_1^z$, which suggests that compactness has already been achieved in B and the transformation of B to C is only the replacement of another aqua ligand through chelation. For aqua ligand substitution reactions on cis-[Pt(cis-dach)(H$_2$O)$_2$]$^{2+}$ complex with different donor ligands has different enthalpy of activation ($\Delta H^z$) and entropy of activation ($\Delta S^z$) depending on their ligand character. For the system cis-[Pt(cis-dach)-(H$_2$O)$_2$]$^{2+}$ the enthalpy of activation ($\Delta H_1^z$) values are 32.9, 36.1 and 43.1 kJmol$^{-1}$ for ligands glutathione, DL-penicillamine, and adenosine, respectively (Table 4.3). This is because the ligand adenosine has higher enthalpy of activation due to its weak ability to form hydrogen bond during substitution (outer sphere association) with leaving aqua ligand, whereas for glutathione and DL-penicillamine, before substitution, they are able to form hydrogen bond with aqua ligand by –SH group, which finally leads to Pt-S bond at the same time; Pt(II) has greater affinity for S donor centre in comparison with N donor centre because of soft-soft interaction. DL-penicillamine has higher $\Delta H_1^z$ in comparison to glutathione as the –S-H group of DL-penicillamine is sterically hindered by two methyl groups, which makes DL-penicillamine a weaker ligand in comparison to glutathione.
Figure 4.13: Plausible mechanism for the substitution of the aqua ligand from cis-[Pt(cis-dach)(H₂O)₂]²⁺ by adenosine.
References


Chapter 5

*Kinetic studies on interaction of platinum(II) complexes with ‘S’ containing ligand in aqueous medium*
5.1 Introduction

Platinum chemistry has considerable importance in medicine and biochemistry. Platinum has served the medical world in the form of cis-diamminedichloroplatinum(II) (cis-DDP), which has had notable success as an anticancer drug [1-5]. The interest in platinum-based antitumor drugs has its origin in the 1960s, with the serendipitous discovery by Rosenberg, of the inhibition of cell division by platinum complexes [6]. However its applicability is still limited to a relatively narrow range tumors and its clinical use is reduced by undesirable side effects [7, 8]. In the search for new platinum anticancer drugs, great efforts were devoted to design complexes more efficient and less toxic than reference drugs already in clinical use. For this purpose, the rational design of complexes and the studies on relevant structure-activity relationship have been extended to families of new compounds having high structural diversity. Thus investigations being carried out to synthesize second generation drugs with improved toxicological profile and third generation drugs to overcome cisplatin resistance [9].

Interactions between platinum(II) complexes and ‘S’ bonding ligands are very important from biological and bioinorganic point of view and have attracted considerable interest in recent years [10, 11]. Sulfur containing molecules have a high affinity for platinum(II); examples of reactive biomolecules include cysteine, methionine, glutathione and proteins. Endogenous and exogenous sulfur-containing molecules play a significant role in the metabolism of platinum-based antitumor complexes. Binding of cis-diamminedichloroplatinum(II) (cisplatin or cis-DDP), the antitumor drug, to intracellular thiol groups is known to be the reason for its renal toxicity and other side effects. Reaction with SH groups of protein side chains (e.g., in metallothionein and glutathione, GSH) is thought to trap and deactivate the drug before it reaches its cellular target DNA to form the 1,2 intrastrand cross-link of guanine bases, the likely cytotoxic adduct [12]. On the other hand, the platinum-sulfur interactions can be used to produce favorable effects in the clinical application of Pt-based drugs. It is possible now to employ sulfur containing compounds as chemo-protectants to mitigate the severe toxic side effects of platinum drugs and some of them have been registered in a number of European countries [13]. The Pt-S(thiol) bond can be terminated in the presence of compounds known as
"rescue agents", which are compounds with sulphur and they are very strong nucleophiles (diethylidithiocarbamate, thiourea, thiosulfate, GSH, cysteine, biotin, etc.). Moreover, the design of new generations of platinum-based drugs can be benefited from the understanding of these interactions [14].

At the same time thiourea and its derivatives have found extensive applications in the fields of medicinal chemistry. They are known to exhibit a wide variety of biological activities such as antiviral, antibacterial, antifungal and so on [15]. Thiourea and its derivatives are also a very useful nucleophile since it combines the ligand properties of thiolates (σ-donor) and thioethers (σ-donor, π-acceptor).

The investigation was attempted to obtain a better understanding of the interaction between Pt(II) complexes and N,N′-diethylthiourea (detu) as sulfur containing ligand. This chapter describes the detailed kinetic and mechanistic studies of aqua ligand substitution from cis-diaquaethylenediamineplatinum(II) and cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) by N,N′-diethylthiourea in aqueous solution at pH 3.0. At pH 4.0 these reactions are very fast and can not be monitored by usual spectrophotometric method but pH 3.0 is amenable condition for the study and detu is used for the prediction of reactivity pattern of different ‘S’ containing ligands.

5.2 Experimental

The reactant complex cis-[Pt(cis-dach)(OH$_2$)$_2$](ClO$_4$)$_2$ (1) was prepared and characterized as described in previous chapter (Section 2.2). Cis-[Pt(en)(H$_2$O)$_2$]$^{2+}$ (2) complex was also prepared according to the literature method [16] and characterized spectroscopically [17] ($\lambda_{\text{max}} = 256$ nm). The pH of the solution was maintained at (3.0), so that the complex ions exist as diaqua species. The product of the reaction between (1) or (2) with N,N′-diethylthiourea was prepared by mixing the reagents at pH 3.0 in different molar ratios, namely 1:2, 1:3, 1:4, 1:5 and 1:10 and maintaining the mixtures at the constant temperature of 60° C for 48 hours. The absorption spectra of the resulting solutions were recorded and all were found to exhibit almost identical absorbance at 264 nm wavelength. The spectral difference between the product complexes and the substrate complexes are shown in Figure 5.1.
Figure 5.1: Spectral difference between reacting complexes (1 or 2) with N,N'-diethylthiourea substituted product (3 or 4); For (1 or 2), [complex] = 1.50 × 10⁻⁴ mol dm⁻³; and for (3 or 4) [complex] = 1.50 × 10⁻⁴ mol dm⁻³ and [N,N'-diethylthiourea] = 3.00 × 10⁻³ mol dm⁻³ respectively, cell used = 1 cm quartz, pH = 3.0, and ionic strength = 0.1 mol dm⁻³ NaClO₄.

The composition of the products in the reaction mixtures was determined by Job’s method of continuous variation (Figure 5.2). The metal: ligand ratio was found to be 1:2.

Figure 5.2: Job’s plot for the reaction of N,N'-diethylthiourea with complex (1) and complex (2) at pH = 3.0, and ionic strength = 0.1 mol dm⁻³ NaClO₄.
Cis-[Pt(cis-dach)(OH$_2$)$_2$]$^{2+}$ and cis-[Pt(en)(H$_2$O)$_2$]$^{2+}$ were mixed separately with N,N'-diethylthiourea in 1:2 molar ratio at pH 3.0 and brown products were obtained on slow evaporation. The IR spectrum of N,N'-diethylthiourea shows a band at 3021 cm$^{-1}$ assigned to $\nu$(NH), but does not show any band around 2500 cm$^{-1}$ attributable to $\nu$(S–H) of thiol tautomer, indicating that N,N'-diethylthiourea exists in the thione form in the solid state. Two bands at 1246 and 797 cm$^{-1}$ attributable to $\nu$(CS) vibrations and two strong bands at 2871 and 2933 cm$^{-1}$ assignable to $\nu$(CH) of the –CH$_3$ and –CH$_2$ groups respectively. The strong absorption band at 1093 cm$^{-1}$ is greatly eliminated when N,N'-diethylthiourea is complexed with Pt(II) [18]. This is explained as, due to considerable change in the nature of the N-C bond and the C=S bond on coordination with N,N'-diethylthiourea through the sulfur atom; the N-C-N stretching frequency is increased and the C=S stretching frequency is decreased. However, since the symmetric N-C-N stretching vibration cannot contribute much to the band intensity, the result is a decrease in intensity and a shift in frequency because of reduced double-bond character of C=S. The lowering of frequency in the 670 cm$^{-1}$ region also attributed to the reduced double-bond character of C=S. This is evident that the N,N'-diethylthiourea is coordinated to Pt(II) in the two types of complexes via thione-S. This is further supported by the appearance of new weak bands in Pt(II) complexes in the region ~358 cm$^{-1}$, assignable to $\nu$(Pt–S) [19]. The spectra of both the complexes suggest that the final products are ‘S’ coordinated and the N,N'-diethylthiourea behaves as a monodentate ligand in the experimental pH.

The aqueous solutions of cis-[Pt(cis-dach)(OH$_2$)$_2$]$^{2+}$ and N,N'-diethylthiourea were mixed in a 1:1 molar ratio and the mixture was thermo stated at 60 °C for 48 hours and used for ESI-MS measurement. The ESI mass spectrum of the resulting solution is shown in Figure 5.3. It is clear from this spectrum that the ion at m/z 673.19 (major peak) has become the precursor ion species in the mixture solution and this is tentatively attributed to [2(N,N'-diethylthiourea) + Pt(II) + dach + ClO$_4$]$^+$. The relative abundance of the isotopic peaks matched the expected values very well, i.e., m/z 672.17 ~ 32%, m/z 673.19 ~ 34%, m/z 674.18 ~ 25% [20]. Therefore, the m/z values and isotopic
distribution pattern confirmed that the product is N,N'-diethylthiourea substituted complex.

**Figure 5.3:** ESI-mass spectrum of the product for complex (1) and the N,N'-diethylthiourea substituted product at pH 3.0 in aqueous medium and at ionic strength 0.1 mol dm$^{-3}$ NaClO$_4$. 
Quantum chemical calculations were performed to gain more insight into this process. The applied level (B3LYP/LANL2DZ) shows, as expected, a good geometrical correlation between calculated (Figure 5.4) and experimental structure (Table 5.1) [21, 22]. Amongst all the optimized structures of the examined species, no imaginary wave number mode was obtained, providing that a true minimum on the potential surface was found.

Table 5.1: Geometrical parameters (DFT) of N,N'-diethylthiourea, complex (I), intermediate species (IS) and product, atoms labeling according to Figure 5.4.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Bond length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N,N'-diethylthiourea</td>
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</tr>
<tr>
<td>2C-S1</td>
<td>1.7489</td>
</tr>
<tr>
<td>2C-N4</td>
<td>1.3763</td>
</tr>
<tr>
<td>2C-N3</td>
<td>1.3763</td>
</tr>
<tr>
<td>Complex (I)</td>
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</tr>
<tr>
<td>Pt-N4</td>
<td>2.0365</td>
</tr>
<tr>
<td>Pt-N5</td>
<td>2.0414</td>
</tr>
<tr>
<td>Pt-O2</td>
<td>2.1160</td>
</tr>
<tr>
<td>Pt-O3</td>
<td>2.1163</td>
</tr>
<tr>
<td>IS</td>
<td></td>
</tr>
<tr>
<td>Pt-N2</td>
<td>2.0430</td>
</tr>
<tr>
<td>Pt-N3</td>
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<tr>
<td>Pt-O2</td>
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</tr>
<tr>
<td>Pt-S5</td>
<td>2.4405</td>
</tr>
<tr>
<td>Product</td>
<td></td>
</tr>
<tr>
<td>Pt-N3</td>
<td>2.1028</td>
</tr>
<tr>
<td>Pt-N2</td>
<td>2.1028</td>
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<tr>
<td>Pt-S4</td>
<td>2.4463</td>
</tr>
<tr>
<td>Pt-S24</td>
<td>2.4463</td>
</tr>
</tbody>
</table>
Figure 5.4: Optimized structures of the [Pt(dach)(H₂O)₂]²⁺ complex, N,N’-diethylthioureia, intermediate state (IS) and final product complex calculated at B3LYP/LANL2DZ level.
Figure 5.5: $^1$H NMR spectrum of N,N$'$/diethylthiourea.
In N,N'-diethylthiourea there is three types of protons (Figure 5.5): 4.7 ppm (sharp and well integrated, -NHCH$_2$- proton), 3.35 ppm (-NH-) and 1.1 ppm (-CH$_3$). The $^1$H NMR of the N,N'-diethylthiourea substituted platinum complex (Figure 5.6), showed that the peak of the –NH- proton was not shifted significantly. The minor changes of the peak in position and sharpness is associated with protons attached to - CH$_2$- carbon center from 4.7 to 4.8 ppm (in Pt complex) definitely indicate the coordination of the N,N'-diethylthiourea to the platinum(II) center. This clearly indicates that the –NH- group of N,N'-diethylthiourea remains unaffected during complexation process. Pt(II) is likely to be coordinated to the sulfur atom considering the strong coordination ability of this functional group [23]. Thus the structure proposed here for product ion species, deduced from ESI-mass spectrum for complex (I), is consistent with the other experimental findings.

5.3 Physical measurements
All the spectroscopic scanning and kinetic measurements were done on a Shimadzu UV-Vis spectrophotometer (UV-2450). IR Spectra (KBr disc, 4000 – 350 cm$^{-1}$) were measured in Perkin-Elmer FTIR model RX1 Infrared spectrophotometer. ESI-mass
spectrum was recorded using a micromass Q-Tof micro™ mass spectrometer in +ve ion mode. $^1$H NMR spectra were recorded using Bruker AVANCE III 400 MHz spectrometer. The pHs of the solutions were adjusted by adding NaOH/HClO$_4$, and the measurements were carried out with the help of a Sartorius Digital pH meter (model PB11) with an accuracy of ± 0.01 units. Doubly distilled water was used to prepare all the solutions. All other chemicals used were of 98% pure and obtained from Sigma Chemical Co. The reactions were carried out at constant ionic strength (0.1 mol dm$^{-3}$ NaClO$_4$).

5.4 Kinetic measurements

The kinetic studies were done on a Shimadzu UV 2450 spectrophotometer attached to a thermoelectric cell temperature controller (model TCC-240A with an accuracy of ± 0.1 ºC). The absorption due to N,N’-diethylthiourea was subtracted by using 1:1 (molar ratio) ligand : water mixture in the reference cell. The absorbance at 264 nm was monitored to follow the progress of the reaction. Conventional mixing technique was followed and pseudo first order conditions with respect to metal ion concentration, were maintained throughout the course of the reaction. The plots of ln ($A_\infty - A_t$) (where $A_t$ and $A_\infty$ are absorbances at time t and after completion of reaction respectively) against time (t) (Figure 5.7) were found to be nonlinear; they are curved at initial stage and subsequently of constant slope. This indicates that the reactions are not a single step process. Here it is assumed to be a two step consecutive process, both the steps being dependent upon ligand concentration. From the limiting linear portion of ln ($A_\infty - A_t$) versus time (t) curves, $k_{2(\text{obs})}$ values were obtained. The $k_{1(\text{obs})}$ values were obtained from the plots of ln$\Delta$ versus time (t) where time (t) is small (Figure 5.8). Origin software was used for computational works. Rate data, represented as an average of duplicate runs, are reproducible within ± 4%.
Figure 5.7: A typical kinetic plot of $\ln(A_\infty - A_t)$ versus time (t). [complex (I)] = $1.5 \times 10^{-4}$ mol dm$^{-3}$; [N,N$'$-diethylthiourea] = $3.0 \times 10^{-3}$ mol dm$^{-3}$; pH = 3.0; $\mu = 0.1$ mol dm$^{-3}$ NaClO$_4$ and Temperature = 30 °C.

Figure 5.8: A typical kinetic plot of $\ln(\Delta)$ versus time (t). [complex (I)] = $1.5 \times 10^{-4}$ mol dm$^{-3}$; [N,N$'$-diethylthiourea] = $3.0 \times 10^{-3}$ mol dm$^{-3}$; pH = 3.0; $\mu = 0.1$ mol dm$^{-3}$ NaClO$_4$ and Temperature = 35 °C.

5.5 Results and discussion
The pK$_1$ of N,N$'$-diethylthiourea is 2.91 at 25 °C [24]. Therefore at pH 3.0 the species involved in the kinetic process are the neutral and protonated (~45%) form of N,N$'$-diethylthiourea. The pK$_1$ and pK$_2$ [15] for cis-diaquaethylenediamineplatinum(II) are 5.8 and 7.6 respectively and pK$_1$ and pK$_2$ (6.25 and 7.80) for cis-(diaqua(cis-1,2-diaminocyclohexane)platinum(II) have been evaluated by Irving–Rossotti titration technique [25]. Therefore, it can be assumed that at pH 3.0 all the reactants exist as the diaqua ion. A comparison with the values for diaqua form of complexes (I) and (2) shows a increase in the pK$_1$ values with the increasing $\sigma$ donor ability of the ligands: NH$_2$CH$_2$R < NH$_2$CHR$_2$ (in the corresponding complexes (2) < (I)). From pK values of complexes it can be concluded that the complex (2) contains lower electron density on
Pt(II) centre. The substitution reaction involves a two-step consecutive process; in the first step, one aqua ligand is replaced from cis-[Pt(cis-dach)(OH₂)₂]²⁺ by N,N'-diethylthiourea. The second step is slower, where another aqua ligand is substituted with the second N,N'-diethylthiourea. The rate constant for such a process can be evaluated by assuming the following scheme:

\[ A \xrightarrow{k_1} B \xrightarrow{k_2} C \]

Where A is the diaqua species (I); B is the single substituted intermediate, and C is the final product (3), cis-[Pt(cis-dach)(N,N'-diethylthiourea)₂]²⁺. Formation of C from B is predominant after some time has elapsed. Similar scheme is fitted for complex (2) and the final product is (4), cis-[Pt(en)(N,N'-diethylthiourea)₂]²⁺.

5.6 Calculation of \( k_1 \) for A→B step

The rate constant \( k_{1(o bs)} \) for the A→B step can be evaluated by the method of Weyh and Hamm [26] as discussed in chapter 2 (Section 2.6).

\( k_{1(o bs)} \) is derived from the slope of ln\( \Delta \) versus time(t) plot (correlation coefficient 0.998), when time (t) is small (Figure 5.8). A similar procedure is applied for each ligand in the concentration range of 0.0015 – 0.003 mol dm⁻³ at constant complex (I) concentration of 0.00015 mol dm⁻³ at pH 3.0 and at 20, 22.5, 25, 30 and 35 °C respectively. The \( k_{1(o bs)} \) values thus obtained are linearly dependent on ligand concentration over the studied concentration range. The \( k_{1(o bs)} \) values for different ligand concentrations at different temperatures are given in Table 5.2. The ligand concentration dependence of \( k_{1(o bs)} \) values can be explained by considering the following scheme 5.1.

\[
\begin{align*}
\text{A} & \xrightarrow{k_1} \text{B} \xrightarrow{k_2} \text{C} \\
\text{[Pt(dach)(H₂O)₂]²⁺ + detu} & \xrightleftharpoons[k_1]{\text{anotation}} \text{[Pt(dach)(H₂O)(detu)]²⁺ + H₂O} \\
\text{B} & \xrightarrow{k_2} \text{C} \\
\text{[Pt(dach)(H₂O)(detu)]²⁺ + detu} & \xrightarrow[k_2]{\text{anotation}} \text{[Pt(dach)(detu)₂]²⁺ + H₂O} \\
\end{align*}
\]

Scheme 5.1

Where detu is the neutral form of N,N'-diethylthiourea ligand.

Based on the above scheme and incorporating protonated form of detu a rate expression (5.5) can be derived for the A → B step:

\[
dB/dt = [k_1/(1 + [H⁺]/K₁)] [cis-Pt(cis-dach)(H₂O)₂]²⁺ [N,N'-diethylthiourea]_{total} \]

(5.4)
Where \([\text{cis-Pt(cis-dach)(H}_2\text{O})_2^{2+}]_{\text{total}}\) is the total concentration of the platinum complex and \([\text{N,N'-diethylthiourea}]_{\text{total}} = [\text{N,N'-diethylthiourea}](1 + [H^+/K_1])\); at pH 3.0 where \(K_1\) is the acid dissociation constant of protonated N,N'-diethylthiourea. Hence it can be written:

\[
k_{1(\text{obs})} = \frac{k_1}{(1 + [H^+/K_1])} [\text{N,N'-diethylthiourea}] \tag{5.5}
\]

where \(k_1\) is the second order rate constant for first step aqua ligand substitution. However, the possibility of interaction between protonated ligand and the positively charged Pt(II)-complex is likely to be minimum. A plot of \(k_{1(\text{obs})} \ (s^{-1}) \) versus \([\text{N,N'-diethylthiourea}]\) should be linear, passing through origin. This was found to be so at all temperatures studied (Figures 5.9 and 5.10). The second-order rate constants \(k_1\) calculated from the slopes of \(k_{1(\text{obs})} \ (s^{-1}) \) versus \([\text{N,N'-diethylthiourea}]\) (mol dm\(^{-3}\)) plots at different temperatures are given in Table 5.3. By reducing the positive inductive effect of the diaminocyclohexane ring in complex (1) on going to the complex(2), an enhancement was observed for the first substitution step \(k_{1(\text{obs})}\) in the reaction with detu.

![Figure 5.9: Plot of \(k_{1(\text{obs})} \ (s^{-1}) \) versus \([\text{N,N'-diethylthiourea}]\) at different temperatures and pH = 3.0. A = 20 °, B = 22.5 °, C = 25 °, D = 30 ° and E = 35 °C for complex (1).](image)

### 5.7 Calculation of \(k_2\) for the B→C step

A linear dependence of the observed rate constants on the detu concentration was found as shown for the complex (1) in Figure 5.11. \(k_{2(\text{obs})}\) can be expressed by equations 5.6.

\[
k_{2(\text{obs})} = \frac{k_2}{(1 + [H^+/K_1])} [\text{N,N'-diethylthiourea}] \tag{5.6}
\]
where $k_2$ is the second order rate constant for second aqua ligand substitution step.

Second N,N'-diethylthiourea now attacks the platinum(II) centre. The rate constants ($k_2$) calculated from the slopes of the $k_{2\text{obs}}$ (s$^{-1}$) versus [N,N'-diethylthiourea] (mol dm$^{-3}$) at different temperatures (Figures 5.11 and 5.12) and are collected in Table 5.4. The steric hindrance of coordinated N,N'-diethylthiourea makes it less electrophilic; hence this step is slower and dependent on ligand concentration variation.

**Table 5.2:** $10^3 k_{1\text{obs}}$ (s$^{-1}$) values for different concentrations of N,N'-diethylthiourea at different temperatures. [Complex (I) and (2)] = $1.5 \times 10^{-4}$ mol dm$^{-3}$, pH = 3.0, $\mu = 0.1$ mol dm$^{-3}$ NaClO$_4$.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>10$^3$ [N,N'-diethylthiourea] (mol dm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.500</td>
</tr>
<tr>
<td>Complex (I)</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>2.52 ± 0.01</td>
</tr>
<tr>
<td>22.5</td>
<td>3.22 ± 0.02</td>
</tr>
<tr>
<td>25.0</td>
<td>4.23 ± 0.01</td>
</tr>
<tr>
<td>30.0</td>
<td>6.13 ± 0.02</td>
</tr>
<tr>
<td>35.0</td>
<td>9.44 ± 0.02</td>
</tr>
<tr>
<td>Complex (2)</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>2.73 ± 0.01</td>
</tr>
<tr>
<td>22.5</td>
<td>3.55 ± 0.02</td>
</tr>
<tr>
<td>25.0</td>
<td>4.75 ± 0.02</td>
</tr>
<tr>
<td>30.0</td>
<td>6.97 ± 0.02</td>
</tr>
<tr>
<td>35.0</td>
<td>9.65 ± 0.02</td>
</tr>
</tbody>
</table>

**Table 5.3:** $k_1$ and $k_2$ values for the substitution reactions of complex (I) and (2).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$k_1$ (dm$^3$ mol$^{-1}$ s$^{-1}$)</th>
<th>$10^2$ × $k_2$ (dm$^3$ mol$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>1.68 ± 0.002</td>
<td>0.64 ± 0.002</td>
</tr>
<tr>
<td>22.5</td>
<td>2.14 ± 0.001</td>
<td>0.83 ± 0.001</td>
</tr>
<tr>
<td>25.0</td>
<td>2.82 ± 0.002</td>
<td>1.10 ± 0.018</td>
</tr>
<tr>
<td>30.0</td>
<td>4.08 ± 0.001</td>
<td>1.55 ± 0.002</td>
</tr>
<tr>
<td>35.0</td>
<td>6.30 ± 0.017</td>
<td>2.64 ± 0.001</td>
</tr>
<tr>
<td>Complex (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>1.82 ± 0.002</td>
<td>0.75 ± 0.002</td>
</tr>
<tr>
<td>22.5</td>
<td>2.36 ± 0.019</td>
<td>0.99 ± 0.001</td>
</tr>
<tr>
<td>25.0</td>
<td>3.16 ± 0.002</td>
<td>1.37 ± 0.001</td>
</tr>
<tr>
<td>30.0</td>
<td>4.64 ± 0.002</td>
<td>1.93 ± 0.001</td>
</tr>
<tr>
<td>35.0</td>
<td>6.43 ± 0.003</td>
<td>2.82 ± 0.002</td>
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</table>
Table 5.4: $10^5 k_{2(\text{obs})}$ (s$^{-1}$) values for different concentrations of N,N'-diethylthiourea at different temperatures. [Complex (1) and (2)] = 1.5 × 10$^{-4}$ mol dm$^{-3}$, pH = 3.0, $\mu$ = 0.1 mol dm$^{-3}$ NaClO$_4$.

<table>
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<tr>
<th>Temp. (°C)</th>
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<th>2.250</th>
<th>2.625</th>
<th>3.000</th>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>0.96 ± 0.01</td>
<td>1.21 ± 0.01</td>
<td>1.44 ± 0.02</td>
<td>1.68 ± 0.02</td>
<td>1.98 ± 0.01</td>
</tr>
<tr>
<td>22.5</td>
<td>1.25 ± 0.02</td>
<td>1.56 ± 0.02</td>
<td>1.88 ± 0.01</td>
<td>2.19 ± 0.03</td>
<td>2.50 ± 0.02</td>
</tr>
<tr>
<td>25.0</td>
<td>1.68 ± 0.02</td>
<td>2.11 ± 0.03</td>
<td>2.53 ± 0.02</td>
<td>2.94 ± 0.04</td>
<td>3.36 ± 0.04</td>
</tr>
<tr>
<td>30.0</td>
<td>2.32 ± 0.01</td>
<td>2.91 ± 0.03</td>
<td>3.48 ± 0.03</td>
<td>4.06 ± 0.03</td>
<td>4.64 ± 0.03</td>
</tr>
<tr>
<td>35.0</td>
<td>3.96 ± 0.02</td>
<td>4.95 ± 0.03</td>
<td>5.94 ± 0.03</td>
<td>6.93 ± 0.04</td>
<td>7.92 ± 0.04</td>
</tr>
<tr>
<td>Complex (2)</td>
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<td></td>
</tr>
<tr>
<td>20.0</td>
<td>1.12 ± 0.02</td>
<td>1.41 ± 0.02</td>
<td>1.68 ± 0.01</td>
<td>1.96 ± 0.03</td>
<td>2.24 ± 0.03</td>
</tr>
<tr>
<td>22.5</td>
<td>1.49 ± 0.02</td>
<td>1.86 ± 0.02</td>
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<td>25.0</td>
<td>2.06 ± 0.01</td>
<td>2.58 ± 0.03</td>
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<td>4.12 ± 0.04</td>
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<td>2.90 ± 0.03</td>
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<td>35.0</td>
<td>4.23 ± 0.03</td>
<td>5.29 ± 0.04</td>
<td>6.34 ± 0.05</td>
<td>7.40 ± 0.04</td>
<td>8.46 ± 0.05</td>
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</tbody>
</table>

5.8 Effect of pH on reaction rate

The reaction was studied at five different pH values. The $k_{\text{obs}}$ values are found to increase with increase in pH in the studied pH range. At a fixed 0.00015 mol dm$^{-3}$ [complex(1)], 0.003 mol dm$^{-3}$ [N,N'-diethylthiourea] and 0.1 mol dm$^{-3}$ ionic strength the $10^5 k_{1(\text{obs})}$ values at 30 °C in aqueous medium are 2.42, 5.23, 9.67 12.26 and 18.39 s$^{-1}$ and $10^5 k_{2(\text{obs})}$ values are 0.96, 2.01, 3.68, 4.64 and 11.54 s$^{-1}$ at pH 2.0, 2.4, 2.8, 3.0 and 3.6 respectively. The enhancement in rate may be explained by the acid dissociation equilibrium of the ligand and the complex. With increasing pH, the proportion of the more reactive detu form increases over that of detuH$, \text{H}_2$, which accounts for the increase in rate with increasing pH. The complex also changes its form, from aqua to hydroxoaqua. The hydroxo species is more reactive due to the well-known labilising effect of the $\text{H}_2$ group via its $\text{p}$-bonding ability and strong electromeric effect. Similar trends in rate constants for both the steps were also observed in case of complex 2.
5.9 Effect of temperature on reaction rate

The reactions for both the complexes were studied at five different temperatures for different ligand concentrations and the anation rate constants for both A→B (k₁) and B→C (k₂) steps are given in Table 5.3. The activation parameters calculated from Eyring plots (Figures 5.13, 5.14, 5.15 and 5.16) are given in Table 5.5 and compared with those for analogous systems involving the substitution in square planar platinum(II) complexes.

Table 5.5: Activation parameters for analogous systems

<table>
<thead>
<tr>
<th>Systems</th>
<th>ΔH₁</th>
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<th>ΔH₂</th>
<th>ΔS₂</th>
<th>Refs.</th>
</tr>
</thead>
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<td>Cis-[Pt(en)₂(H₂O)₂]²⁺</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>/Pyridine-2-thiol</td>
<td>36 ± 3</td>
<td>-166 ± 8</td>
<td>26 ± 1</td>
<td>-195 ± 4</td>
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<tr>
<td>/Thiosemicarbazide</td>
<td>35.6 ± 0.8</td>
<td>-166 ± 3</td>
<td>44.5 ± 1.3</td>
<td>-182 ± 4</td>
<td>[28]</td>
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<tr>
<td>/N,N'-diethylthiourea 60.4 ± 3.8</td>
<td>-33 ± 13</td>
<td>62.7 ± 4.5</td>
<td>-71 ± 15</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>Cis-[Pt(cis-dach)(H₂O)₂]²⁺</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>/Glutathione</td>
<td>32.9 ± 1.3</td>
<td>-187.2 ± 4.2</td>
<td>30.5 ± 0.1</td>
<td>-223.1 ± 0.3</td>
<td>[29]</td>
</tr>
<tr>
<td>/Diethyldithio-carbamate</td>
<td>66.8 ± 3.7</td>
<td>-81 ± 12</td>
<td>95.1 ± 2.8</td>
<td>-34.4 ± 9.1</td>
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<tr>
<td>/N,N'-diethylthiourea 62.8 ± 2.1</td>
<td>-26 ± 7</td>
<td>66.2 ± 3.4</td>
<td>-61 ± 11</td>
<td></td>
<td>**</td>
</tr>
</tbody>
</table>

** This work

5.10 Mechanism and conclusion

The present investigation of substitution of aqua ligands in cis-diaqua(cis-1, 2-diaminocyclohexane)platinum(II) ion by N,N'-diethylthiourea shows that N,N'-diethylthiourea interacts in an associative mode of activation in the transition state. The second step is slower than the first step and also dependent on ligand concentration. The ligand N,N'-diethylthiourea exists as a mixture of neutral and protonated form at experimental pH 3.0. However, the possibility of the interaction between the protonated ligand and the positively charged Pt(II) complexes may be ruled out. The sulfur end of N,N'-diethylthiourea is a soft donor centre and has a large affinity for the soft Pt(II) centre. The results of Job’s method of continuous variation indicate 1:2 molar ratio and the IR spectra of the solid products suggest that N,N'-diethylthiourea behaves as a
monodentate ligand binding through ‘S’ end. Finally ESI-mass spectrum and DFT studies provide a qualitative picture of the composition of the product i.e. the ligational sites of N,N′-diethylthiourea, bond lengths and bond angles of the same. Thus the mechanism of substitution of aqua ligands can be explained in terms of two consecutive steps; both the steps are dependent of ligand concentration, and the second step is slower than the first step (Figure 5.17).

The activation parameters suggest an associative mode of activation for the substitution processes. The enthalpy of activation (\(\Delta H^\ddagger_1\) and \(\Delta H^\ddagger_2\)) values and negative entropy of activation (\(\Delta S^\ddagger_1\) and \(\Delta S^\ddagger_2\)) values indicate N,N′-diethylthiourea participation in the transition state. The positive enthalpy change for breaking the M-OH\(_2\) bond is partially compensated by the formation of M-L bond in the transition state. The participation of N,N′-diethylthiourea in the transition state results in a more compact state and negative \(\Delta S^\ddagger\) value is obtained. Further \(\Delta S^\ddagger_2\) is more negative than \(\Delta S^\ddagger_1\), which suggest that compactness has already been achieved in B and the transformation of B to C is the replacement of another aqua ligand through insertion of second ligand. For aqua ligand substitution reactions between complexes (1) or (2) with sulphur donor ligand give different enthalpy of activation (\(\Delta H^\ddagger\)) and entropy of activation (\(\Delta S^\ddagger\)) depending on the complex. The trends in the reactivity of the complexes can also be seen in the activation enthalpies for the reactions involving the nucleophile N,N′-diethylthiourea. The higher the electrophilicity of the metal centre, the smaller the activation enthalpy because of the better participation of the ligand in the transition state (viz., \(\Delta H^\ddagger_1(Pt(en)) = 60.4 \pm 3.8 \text{ kJ mol}^{-1}\), \(\Delta H^\ddagger_1(Pt(dach)) = 62.8 \pm 2.1 \text{ kJ mol}^{-1}\)). Thus the reactivity order of the Pt(II) aqua complexes (viz., Pt(dach) < Pt(en)) conforms to which was already expected on the basis of the pKa values. The variation in pKa of the N,N′-diethylthiourea with temperature was not taking into account as those are not available from literature, which may also affect the rate constants. So activation parameters were not correlated with the ligand acid dissociation constant.
Figure 5.10: Plots of $k_{1\text{obs}}(\text{s}^{-1})$ versus $[\text{N,N'-diethylthiourea}]$ at different temperatures and pH = 3.0. A = 20 °C, B = 22.5 °C, C = 25 °C, D = 30 °C and E = 35 °C for complex (2).

Figure 5.11: Plots of $k_{2\text{obs}}(\text{s}^{-1})$ versus $[\text{N,N'-diethylthiourea}]$ at different temperatures; A = 20 °C, B = 22.5 °C, C = 25 °C, D = 30 °C and E = 35 °C for complex (1); pH = 3.0 and ionic strength = 0.1mol dm$^{-3}$ NaClO$_4$. 
Figure 5.12: Plots of $k_{2\text{ (obs)}}$ (s) versus $[\text{N,N'}$-diethylthiourea] at different temperatures; A = 20 °C, B = 22.5 °C, C = 25 °C, D = 30 °C and E = 35 °C for complex (2); pH = 3.0 and ionic strength = 0.1 mol dm$^{-3}$ NaClO$_4$.

Figure 5.13: Eyring plot of $k_1$ for complex (1)
Figure 5.14: Eyring plot of $k_1$ for complex (2)

Figure 5.15: Eyring plot of $k_2$ for complex (I)
Figure 5.16: Eyring plot of $k_2$ for complex (2)

Figure 5.17: Plausible mechanism for the substitution of the aqua ligand from $cis$-[Pt($cis$-dach)(H$_2$O)$_2$]$^{2+}$ by N,N’-diethylthiourea.
References
Chapter 6

Mechanistic and kinetic investigations on the interaction of model platinum(II) complex with ligands of biological significance in reference to the antitumour activity
6.1 Introduction

Platinum coordination complexes are widely used in the treatment of human cancer. Since the discovery of the activity of one of the most successful anticancer compound *cis*-diaminedichloroplatinum(II), \([\text{cis-Pt(NH}_3)_2\text{Cl}_2]\), clinically called cisplatin, thousands of platinum complexes have been synthesized and evaluated for their anticancer activity [1-5]. Despite their tremendous success, these platinum compounds suffer from two main disadvantages: they are inefficient against platinum-resistant tumors, and they have severe side effects (gastrointestinal and kidney toxicity, immune system suppression, peripheral neurotoxicity, etc.) [6]. Furthermore, as a consequence of its particular chemical structure, cisplatin in particular offers little possibility for rational improvements to increase its tumor specificity and thereby reduce undesired side effects [7]. Antitumour activity of platinum complexes is varied by the replacement of their leaving and non-leaving groups. Among the analogues of cis-DDP the platinum complexes which have 1,2-diaminocyclohexane (abbreviated as dach) as the non-leaving amine ligand have been especially noted as second generation of platinum drugs. It has been so also because they lack cross-resistance with established drugs like *cis*-DDP [8].

There are many other potential biomolecules that can also react with this Pt(II) complex, such as small molecules, proteins and enzymes. In fact, already in blood where Pt drug is administered by injection or infusion, several molecules are available for thermodynamic and kinetic competition [9-12]. Binding to DNA eventually leads to an altered protein conformation and changes in biological activity, especially when enzymatic reactions are affected. Some N,N-bound platinum(II) complexes bind to the side chain of methionine, histidine, arginine, and cysteine residue of certain proteins and result in selective hydrolytic cleavage of adjacent amide bond [13]. In view of greater affinity of platinum(II) for nitrogen and sulfur-containing ligands, it is of interest to investigate their reactions with amino acids and peptides.

This chapter describes the binding of the possible anticancer drug *cis*-diaqua(*cis*-1,2-diaminocyclohexane)platinum(II) with different biomolecules (L-asparagine, L-arginine and L-glutamic acid) and the factors (steric or electronic) that influence the affinities of amino acids for the complex. A better understanding of the cellular responses to platinum compounds would aid in the design of novel platinum-based anticancer agents and
suggest strategies for improving the effectiveness of cancer therapy with the existing drugs.

6.2 Experimental

The reactant complex was prepared and characterized as described in previous chapter (Section 2.2). The pH of the solution was maintained at pH (4.0), so that perchlorate salt exists as diaqua species. The products of the reaction between complex (I) and different amino acids [L-asparagine (L$^1$H), L-arginine (L$^2$H) and L-glutamic acid (L$^3$H)] were prepared by mixing the reactants at pH 4.0 in different molar ratios: viz 1:1, 1:2, 1:3, 1:4 and 1:5 and thermostating the mixtures at 60 °C for 48 hours. The absorption spectra of the resulting solutions were recorded and all were found to exhibit almost nearly identical absorbances at 211 nm wavelength. The difference in spectra between the product complex and the substrate complex is shown in Figure 6.1.

Figure 6.1: Spectra of the starting complex (1), L$^1$H substituted complex (2), L$^2$H substituted complex (3) and L$^3$H substituted complex (4); [complex (I)] = 2.00 $\times$ 10$^{-4}$ mol dm$^{-3}$, [amino acids] = 4.00 $\times$ 10$^{-3}$ mol dm$^{-3}$, pH = 4.0, cell used = 1 cm quartz.

The composition of the product in the reaction mixture was determined by Job’s method of continuous variation (Figure 6.2). The metal: ligand ratio was found to be 1:1.
Figure 6.2: Job’s plot for the reaction between complex (I) and \( \text{L}^2\text{H} \) at pH = 4.0 and ionic strength = 0.1 mol dm\(^{-3}\) NaClO\(_4\). 

Cis-\([\text{Pt(cis-dach})(\text{OH}_2)]^{2+}\) and amino acids were mixed in 1:1 molar ratio at pH 4.0 and yellow solids were isolated. The IR spectra of the products in KBr disc show strong bands at \( \sim 3414, 1638 \) (1654 cm\(^{-1}\) for L-arginine substituted complex (I)) and 627 cm\(^{-1}\) together with medium bands at 3237-3100 cm\(^{-1}\) and \( \sim 477 \) cm\(^{-1}\). The presence of strong stretching band at \( \sim 3414 \) cm\(^{-1}\) indicates the product is hydrated. The asymmetric COO\(^{-}\) stretching frequency (\( \nu_{\text{asym}} \)) of the amino acids occurs at 1580–1660 cm\(^{-1}\) when the group is coordinated to metals, whereas a noncoordinated COO\(^{-}\) group has the \( \nu_{\text{asym}} \) (COO\(^{-}\)) stretching at lower frequencies [14]. The band at 1638 and 1654 cm\(^{-1}\) is therefore due to the \( \nu_{\text{asym}} \) (COO\(^{-}\)) of the metal bound carboxyl group. The broad band at 3237-3100 cm\(^{-1}\) is assigned to overlapping of \( \nu_{\text{asym}} \) and \( \nu_{\text{sym}} \) motion of the \(-\text{NH}_2\) group coordinated to platinum(II) in the product complex. The band at \( \sim 477 \) cm\(^{-1}\) is also assigned to \( \nu(\text{Pt-N}) \) bond formation [15]. From the individual assignments of different bands it can be presumed that the final product is a (O, N) coordinated chelate and amino acids behaves like a bidentate ligand in the experimental pH.
Figure 6.3: $^1$H NMR spectra of the L-glutamic acid and L-glutamic acid substituted cis - 
$[\text{Pt}(\text{cis-dach})(\text{OH}_2)_2]^{2+}$ complex.
In glutamic acid there are four types of protons: 4.694 ppm (2H, broad, -NH$_2$ proton), 3.685 ppm (triplet, 1H, NH$_2$-CH-CH$_2$-), 2.45 ppm (2H, triplet, OCCH$_2$-CH$_2$) and 2.0 ppm (2H, multiplet, OCCH$_2$-CH$_2$-CH). The $^1$H NMR of the substituted platinum complex, the peak of the -NH$_2$ proton is not shifted significantly as the –NH$_2$ peak is broad in nature; it is not possible to draw any direct conclusion from this. But the other three peak positions are changed significantly: the major change of position of the peak of the proton attached to C-2 carbon center from 3.685 to 4.15 ppm (in Pt complex) indicates the coordination of the -NH$_2$ and adjacent –CO group to the platinum center. As the two coordinating groups are attached to the C$_2$, the shift of the proton attached to C-2 is large. In case of other peaks, a minor change of the peak positions from 2.45 to 2.65 ppm and 2.0 to 2.3 ppm in substituted Pt(II) complex is observed. This clearly indicates the coordination of the –NH$_2$ and carboxylic acid group of glutamic acid to the metal center while the side chain remains unaffected during complexation process.

The aqueous solutions of cis-[Pt(cis-dach)(OH)$_2$)$_2$]$^{2+}$ and L-glutamic acid were mixed in a 1:1 molar ratio and the mixture was thermo stated at 60 °C for 48 hours and used for ESI-MS measurement. The ESI mass spectrum of the resulting solution is shown in Figure 6.4.
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Figure 6.4: ESI- mass spectrum of the product i.e., L-glutamic acid substituted cis - [Pt(cis-dach)(OH₂)₂]²⁺ complex.

It is clear from this spectrum that the ion at m/z 455.18 has become the precursor ion species in the mixture solution and this is tentatively attributed to [L-glutamic acid + Pt(II) + dach]⁺. Therefore, the m/z value indicates that the product is amino acid substituted complex (I). Thus the structure proposed here for product ion species, deduced from ESI-mass spectrum, is generally consistent with those derived from other experimental methods.

6.3 Physical measurements

All the spectroscopic scanning and kinetic measurements were done in a Shimadzu UV-Vis spectrophotometer (UV-2450) attached to a thermoelectric cell temperature controller (Model TCC-240A with an accuracy of ± 0.1 °C). IR Spectra (KBr disc, 4000 – 300 cm⁻¹) were measured in Perkin-Elmer FTIR model RX1 Infrared spectrophotometer. ESI-mass spectrum was recorded using a micromass Q-Tof micro™ mass spectrometer in +ve ion mode. ¹H NMR spectrum was recorded using Bruker AVANCE III 400 MHz spectrometer. The pHs of the solutions were adjusted by adding NaOH/HClO₄ and the
measurements were carried out with the help of a Sartorius Digital pH meter (model PB11) with an accuracy of ± 0.01 units. Doubly distilled water was used to prepare all the solutions. All other chemicals used were of AR grade. The reactions were carried out at constant ionic strength (0.1 mol dm⁻³ NaClO₄).

6.4 Kinetic measurements

The kinetic studies were done on a Shimadzu UV 2450 spectrophotometer attached to a thermoelectric cell temperature controller (model TCC-240A with an accuracy of ± 0.1 °C). The absorption due to amino acids was subtracted by using 1:1 (molar ratio) ligand:water mixture in the reference cell. The increase in absorbance at 211 nm was monitored to follow the progress of the reaction. Conventional mixing technique was followed and pseudo first order conditions with respect to metal ion concentration, were maintained throughout the course of the reaction. A typical plot of ln (Aᵢ - Aₜ) (where Aᵢ and Aᵢ are absorbances at time (t) and after completion of the reaction) against time (t) (Figure 6.5) is found to be nonlinear; it is curved at initial stage and subsequently of constant slope indicating that the reaction proceeds via two consecutive steps. From the limiting linear portion of ln (Aᵢ - Aₜ) versus time (t) curve, k₂(obs) was obtained. The k₁(obs) values were obtained from the plots of lnΔ versus time (t) where time (t) is small (Figure 6.6). Origin software was used for computational works. Rate data, represented as an average of duplicate runs, are reproducible within ± 4%.

![Figure 6.5](image.png)

**Figure 6.5:** A typical kinetic plot of ln( Aᵢ - Aₜ ) versus time (t). [complex (J)] = 2.0 × 10⁻⁴ mol dm⁻³; [L-asparagine] = 4 × 10⁻³ mol dm⁻³; Temperature = 60 °C and pH = 4.0.
Figure 6.6: A typical kinetic plot of $\ln \Delta$ versus time ($t$). [complex ($I$)] = $2 \times 10^{-4}$ mol dm$^{-3}$; [L-asparagine] = $2 \times 10^{-3}$ mol dm$^{-3}$; Temperature = 60 $^\circ$C and pH = 4.0.

6.5 Results and discussion

The pK$_a$ values of the ligands L$_1^H$, L$_2^H$ and L$_3^H$ are 2.02 (–COOH), 8.80 (NH$_3^+$); 2.17 (–COOH), 9.04 (NH$_3^+$), 12.48 (Guan$^+$) and 2.19 α(–COOH), 4.25 (–COOH) and 9.67 (NH$_3^+$) [16] respectively, at 25 $^\circ$C. From the pK$_a$ values of all the ligands it can be said that at pH = 4.0, all the ligands L$_1^H$, L$_2^H$ and L$_3^H$ remain in the zwitterionic form, which participate in the reaction.

The pK$_1$ and pK$_2$ for cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) have been evaluated by Irving–Rossotti titration technique [17] and found to be 6.25 and 7.80 respectively, it can be assumed that at pH 4.0 the reactant exists as the diaqua ion. At constant temperature and constant pH (4.0) and fixed concentration of complex ($I$), the ln ($A_\infty - A_t$) versus time ($t$) plot for different ligand concentrations are curved at the initial stage and subsequently of constant slope. This indicates that the reaction involves a two-step consecutive process; the first step is dependent on ligand concentration whereas the second one is the independent. In the first step one aqua ligand was replaced from cis-[Pt(cis-dach)(OH$_2$)$_2$]$^{2+}$ by amino acids and in the slower step, where another aqua ligand is substituted, which is a ring closure step. The rate constant for such a process can be evaluated by assuming the following scheme:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$
Where A is the diaqua species (1); B is the single substituted intermediate with monodentate amino acids (LH) and C is the final product (2) \([cis-Pt(cis-dach)(LH)]^+\). Formation of C from B is predominant after some time has elapsed.

### 6.6 Calculation of \(k_1\) for A → B step

The rate constant \(k_{1\text{(obs)}}\) for the A → B step can be evaluated by the method of Weyh and Hamm [18] as discussed in earlier chapter (Section 2.6). \(k_{1\text{(obs)}}\) is derived from the slope of \(\ln \Delta\) versus time(t) plot (correlation coefficient 0.997), when time (t) is small (Figure 6.7). A similar procedure is applied for each ligand in the concentration range of 0.002 – 0.004 mol dm\(^{-3}\) at constant complex (I) concentration of 0.0002 mol dm\(^{-3}\) at pH 4.0 and at 40, 45, 50, 55 and 60 °C respectively. The \(k_{1\text{(obs)}}\) values thus obtained are linearly dependent on the studied concentration range. However, studies at further higher concentration were restricted due to the solubility problem of amino acid molecules in aqueous medium. The \(k_{1\text{(obs)}}\) values for different ligand concentrations at different temperatures are given in Table 6.1. The ligand concentration dependence of \(k_{1\text{(obs)}}\) values can be explained by considering the following scheme 6.1.

\[
\begin{align*}
& [\text{Pt(dach)}(\text{H}_2\text{O})_2]^{2+} + \text{LH} \quad \xrightarrow{k_{1\text{, slow anation}}} \quad [\text{Pt(dach)}(\text{H}_2\text{O})(\text{LH})]^{2+} + \text{H}_2\text{O} \\
& \text{A} \quad \text{B} \\
& [\text{Pt(dach)}(\text{H}_2\text{O})(\text{LH})]^{2+} \quad \xrightarrow{k_2 \text{ chelation}} \quad [\text{Pt(dach)}(\text{L})]^+ + \text{H}_3\text{O}^+ \\
& \text{B} \quad \text{C}
\end{align*}
\]

**Scheme 6.1**

Based on the above scheme a rate expression (6.5) can be derived for the A → B step:

\[
d\text{B}/dt = k_1 [cis-Pt(cis-dach)(\text{H}_2\text{O})_2]_{\text{total}}[\text{amino acids}] \tag{6.4}
\]

Where \([cis-Pt(cis-dach)(\text{H}_2\text{O})_2]_{\text{total}}\) is the concentration of the unreacted complex and \([\text{amino acids}]\) is the concentration of amino acids. Hence it can be written:

\[
k_{1\text{(obs)}} = k_1 [\text{amino acids}] \tag{6.5}
\]

where \(k_1\) is the second order rate constant for the first step of aqua ligand substitution. A plot of \(k_{1\text{(obs)}}\) (s\(^{-1}\)) versus [amino acids] should be linear, passing through the origin. This was found to be so at all temperatures studied (Figure 6.7) for three amino acids. The second-order rate constants (\(k_1\)) calculated from the slopes of \(k_{1\text{(obs)}}\) (s\(^{-1}\)) versus [amino acids] (mol dm\(^{-3}\)) plots at different temperatures are given in Table 6.2. However, the first
order dependence of rate constant on [LH] may also fit with the scheme involving the formation of an outersphere association in TS. The former mechanism is applicable as no evidence of outersphere association complex (i.e. no limiting condition) formation was found within the studied concentration range.

**Table 6.1:** $10^3 k_{1(obs)} (s^{-1})$ values for different concentrations of amino acids at different temperatures. [complex $\{J\} = 2 \times 10^{-4} \text{ mol dm}^{-3}$, pH = 4.0, $\mu = 0.1 \text{ mol dm}^{-3}$ NaClO₄.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Temperature ($\pm 0.1 \text{ °C}$)</th>
<th>$10^3$ [amino acids] (mol dm$^{-3}$)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>2.00</td>
</tr>
<tr>
<td>L$^1$H</td>
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<td>0.73</td>
</tr>
<tr>
<td></td>
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<td>L$^2$H</td>
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<td>L$^3$H</td>
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<tr>
<td></td>
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<td>0.86</td>
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<tr>
<td></td>
<td>60</td>
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Figure 6.7: Plots of $k_{1\text{(obs)}}$ (s$^{-1}$) versus [L-asparagine] at different temperatures.  A = 40 °,  B = 45 °, C = 50 °, D = 55 ° and E = 60 °C

6.7 Calculation of $k_2$ for the B→C step

The B →C step is the ring closure step and is independent of ligand concentration. At a particular temperature the slopes of ln($A_\infty - A_t$) versus time(t) plots for different ligand concentrations were found to be constant in the region where plots are linear (Figure 6.5). For different temperatures the $k_2$ values are obtained directly from the limiting slopes and are collected in Table 6.2. The experimental results show a similar curvature of ln($A_\infty - A_t$) versus time(t) plots at different temperatures for varying concentrations. The assumption of two consecutive steps for such a reaction and the computation of $k_1$ and $k_2$ values fit well with the experimental values.

Table 6.2: $k_1$ and $k_2$ values for the substitution reaction; [complex (I)] = 2.00 × 10$^{-4}$ mol dm$^{-3}$, pH 4.0,  = 0.1 mol dm$^{-3}$NaClO$_4$.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Temperature (±0.1 °C)</th>
<th>$k_1$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</th>
<th>$10^3k_2$ (s$^{-1}$)</th>
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6.8 Effect of temperature on reaction rate

The reaction was studied at five different temperatures for different ligand concentrations and the anation rate constants for both \( A \rightarrow B \) (\( k_1 \)) and \( B \rightarrow C \) (\( k_2 \)) steps are given in Table 6.2. The activation parameters calculated from Eyring plots (Figures 6.8 and 6.9) are given in Table 6.3 and compared with those for analogous systems involving the substitution in square planar platinum(II) complexes.

6.9 Effect of pH on reaction rate

The reaction was studied at four different pH values (pH 2.8, 3.4, 4.0 and 4.6). The \( k_{obs} \) values are found to increase with increase in pH in the studied pH range. The enhancement in rate may be explained based on acid dissociation equilibria of the ligand and complex. With increase in pH, the diaqua complex will be converted into hydroxoaqua complex. The reactivity of hydroxoaqua complex is usually higher than that of diaqua complex by the well-known labilising effect of the coordinated hydroxide ion. On the other hand, with increase in pH deprotonation of the ligand occurs which is also responsible for the enhanced reactivity of the reaction with pH. Notwithstanding in the present kinetic runs, the substitution reactions were followed at a constant pH of 4.0 to avoid complication caused by adding an additional parameter of \([H^+]\) to the rate equation. At pH 4.0 the complex (\( I \)) exists in the diaqua form.
Table 6.3: Activation parameters for analogous systems

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<th>Systems</th>
<th>$\Delta H_1^{\ddagger}$ (kJmol$^{-1}$)</th>
<th>$\Delta S_1^{\ddagger}$ (JK$^{-1}$mol$^{-1}$)</th>
<th>$\Delta H_2^{\ddagger}$ (kJmol$^{-1}$)</th>
<th>$\Delta S_2^{\ddagger}$ (JK$^{-1}$mol$^{-1}$)</th>
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<tr>
<td>Cis-[Pt(cis-dach)(H$_2$O)$_2$]$^{2+}$</td>
<td>32.9 ± 1.3</td>
<td>-187.2 ± 4.2</td>
<td>30.5 ± 0.1</td>
<td>-223.1 ± 0.3</td>
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</tr>
<tr>
<td>/Glutathione</td>
<td>36.1 ± 4.1</td>
<td>-175 ± 12</td>
<td>44.4 ± 1.1</td>
<td>-189 ± 3</td>
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</tr>
<tr>
<td>/DL-penicillamine</td>
<td>51.9 ± 2.8</td>
<td>-152 ± 8</td>
<td>54.4 ± 1.7</td>
<td>-162 ± 5</td>
<td>[21]</td>
</tr>
<tr>
<td>/Glycyl-L-leucine</td>
<td>40.7 ± 1.8</td>
<td>-124 ± 6</td>
<td>44.2 ± 0.3</td>
<td>-191 ± 0.9</td>
<td>[**]</td>
</tr>
<tr>
<td>/L-asparagine</td>
<td>41.5 ± 1.4</td>
<td>-123 ± 4</td>
<td>46.3 ± 0.2</td>
<td>-186 ± 0.6</td>
<td>[**]</td>
</tr>
<tr>
<td>/L-arginine</td>
<td>43.3 ± 1.8</td>
<td>-121 ± 6</td>
<td>49.2 ± 0.1</td>
<td>-177 ± 0.3</td>
<td>[**]</td>
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</table>

** This work

6.10 Mechanism and conclusions

The present investigation of substitution of aqua ligands in cis-diaqua(cis-1, 2-diaminocyclohexane)platinum(II) ion by amino acids shows that the amino acids interact in an associative mode of activation in the transition state. The second step is the chelation i.e. ring closure step, which is slower than the first step and independent of ligand concentration (Figure 6.10). The two cis positions of platinum(II) ion are blocked by NH$_2$ group of dach and in view of preference of square planar configuration in its complexes; it is likely that amino acids behave as a bidentate ligand in the complex formation. The result of Job’s method of continuous variation indicate (1:1 M:L molar ratio) and the IR spectrum of the solid product suggests that amino acids behave as a bidentate ligand. Finally $^1$H NMR and ESI-mass spectra provide a qualitative picture of the composition of the product. The affinity for nitrogen atom of the amino group on amino acids provides the driving force for the ring formation.

The activation parameters for the first and the second step suggest an associative mode of activation for the substitution process (Table 6.3). The enthalpy of activation ($\Delta H_1^{\ddagger}$ and $\Delta H_2^{\ddagger}$) values and negative entropy ($\Delta S_1^{\ddagger}$ and $\Delta S_2^{\ddagger}$) values imply a good degree of ligand participation in the transition state. The positive enthalpy change for breaking the M-OH$_2$ bond is partially compensated by the formation of M-L bond in the transition state. The participation of amino acid in the transition state results in a more compact
state and negative $\Delta S^\neq$ value is obtained. Further, $\Delta S_2^\neq$ is more negative than $\Delta S_1^\neq$, which suggests that compactness has already been achieved in B and the transformation of B to C is only the replacement of another aqua ligand through chelation. For aqua ligand substitution reactions on cis-[Pt(cis-dach)(H$_2$O)$_2$]$^{2+}$ complex with different donor ligands have different enthalpy of activation ($\Delta H^\neq$) and entropy of activation ($\Delta S^\neq$) depending on their ligand character. The rate constant ‘$k_1$’ for the ligands L-asparagine, L-arginine and L-glutamic acid, all having (O, N) donor set and can be rearranged as:

L-glutamic acid (Acidic) < L-arginine (Basic) < L-asparagine (Neutral)

The first step is related with the deprotonation of $-\text{COOH}$ group. So increase in rate from L-glutamic acid to L-asparagine is associated with the pKa values of the ligands.

The chelation reaction also shows different rates for different amino acids. The rate constant for this reaction $k_2$ has been found to be in the order:

L-glutamic acid < L-arginine < L-asparagine

Chelation step is accompanied by deprotonation of the $\alpha$-$\text{NH}_3^+$ of the amino acids. So rate constant values for the second step are in accordance with pKa of $\alpha$-$\text{NH}_3^+$ group.

**Figure 6.8:** Eyring plot for $k_1$ of $L_1^1H$
Figure 6.9: Eyring plot for $k_2$ of $L^1H$

Figure 6.10: Plausible mechanism for the substitution of the aqua ligands from cis-[Pt(cis-dach)(H_2O)_2]^{2+} by amino acids. Where R is H_2N-CO-CH_2- for $L^1H$, NH_2C(=NH)-NH-(CH_2)_3- for $L^2H$ and HOOC-CH_2-CH_2- for $L^3H$
References


Chapter 7

Kinetics and mechanism of some bioactive ligands with cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) perchlorate in aqueous medium
7.1 Introduction

Cisplatin (CDDP) is one of the most effective anticancer drugs currently used worldwide in the treatment of testicular, ovarian, and other tumors [1, 2]. The usefulness of this transition metal complex is limited, however, by its limited spectrum of responsive tumors, rather severe host toxicities such as nausea and vomiting, nephrotoxicity, neurotoxicity and the potential to induce resistance in otherwise responsive tumor types [3]. In addition, cisplatin has been classified as a probable human carcinogen [4]. In the search for cisplatin analogs with a better toxicity profile and an improved spectrum of activity, a large number of platinum (Pt) derivatives has been synthesized and investigated [5-7]. One of the most promising second generation platinum complexes was cis-dichloro(1,2-diaminocyclohexane)platinum(II) [8]. The biological properties of this complex which included excellent antitumor activity, little or no nephrotoxicity and lack of cross resistance with CDDP [8]. Much progress has been made in elucidating its mode of action, and many details of the mechanism by which platinum-based drugs kill cancer cells are now well-established. Biological and chemical experiments have indicated that the interaction of cisplatin with DNA is responsible for its antineoplastic activity [9, 10]. Nevertheless, some essential chemical processes, related to what happens before the cisplatin reaches the DNA, generally considered its final target, are still to be identified. Among these processes, the best known is the formation of aqua species, the main reaction of activation of the drug which occurs in the cytoplasmic compartment by hydrolysis of the chloride ligands. However, many other nongenomic biomolecules could be potential targets for platinum. Sulfur-rich biomolecules, including free amino acids (cysteine and methionine), oligopeptides (glutathione), and proteins represent good targets for a soft metal such as Pt(II) [11-14]. Moreover, the need to improve the cisplatin clinical protocol drives much research into better understanding of its antitumor activity mechanism. On the other hand, in order to overcome acquired cellular resistance to cisplatin, much effort is currently devoted to the discovery of new Pt anticancer drugs. This chapter reports the kinetics of complex formation reaction of cis-[Pt(cis-dach)(H$_2$O)$_2$]$_2^{2+}$ (where ‘dach’ is cis-1,2-diaminocyclohexane) with some biologically relevant nitrogen, oxygen and sulfur donor nucleophiles (2-thiouracil, 1,2-cyclohexanenedionedioxime and acetylacetone). The aqua complex studied is more reactive
than the corresponding chloro complex. The coordinated water molecule on Pt(II) centre has been shown to be very labile and can more easily be substituted than a stronger nucleophilic group such as chloride. These nucleophiles were investigated because of their different nucleophilicity, steric hindrance, binding properties and biological relevance. This study is of fundamental importance to unravel the mode of action of this class of compounds and it throws more light on the kinetic and mechanistic behavior of platinum antitumour complexes. The goal is to contribute to the elucidation of the whole mechanism employed by these compounds to reach the biological target.

7.2 Experimental

The reactant complex was prepared and characterized as described in previous chapter (Section 2.2). The pHs of the solutions were maintained at pH (4.0), so that perchlorate salt exists as diaqua species. The products of the reaction between (I) and 2-thiouracil (L₁H) or 1,2-cyclohexanedionedioxime (L₂H) or acetylacetone (L₃H) were prepared by mixing the reagents at pH 4.0 in different molar ratio: viz 1:1, 1:2, 1:3, 1:4 and 1:5 and thermo stating the mixture at 60 °C for 48 hours. The absorption spectra of the resulting solutions were recorded and all were found to exhibit almost identical absorbance at 327, 298 and 308 nm wavelength for L₁H or L₂H and L₃H respectively. The spectral differences between the product complexes and the substrate complex are shown in Figure 7.1. The relative λ_max positions of the LH substituted products are in accordance with the polarizibility of the ligands.
Figure 7.1: Spectra of the starting complex (1), L$^4$H substituted complex (2) L$^5$H substituted complex (3) and L$^6$H substituted complex (4). [complex (1)] = 2.00 × 10$^{-4}$ mol dm$^{-3}$, [L$^4$H], [L$^5$H] and [L$^6$H] = 4.00 × 10$^{-3}$ mol dm$^{-3}$, cell used = 1 cm quartz, pH = 4.0, and ionic strength = 0.10 mol dm$^{-3}$ NaClO$_4$.

The compositions of the products in the reaction mixtures were determined by Job’s method of continuous variation (Figure 7.2). The metal: ligand ratios were found to be 1:1 for L$^4$H, L$^5$H and L$^6$H.

Figure 7.2: Job’s plot for the reaction between complex (1) and L$^3$H at pH = 4.0 and ionic strength = 0.10 mol dm$^{-3}$ NaClO$_4$. 
Complex (I) and 2-thiouracil (L\textsuperscript{1}H) were mixed in 1:1 molar ratio at pH 4.0 and a yellowish solid was isolated. The IR spectrum of the yellowish product in the KBr disc shows a strong band at 3411 together with medium band at 530 cm\textsuperscript{-1}. The IR spectrum of L\textsuperscript{1}H does not show any \(\nu(\text{SH})\) band, hence in the solid state ligand exist mainly in the keto-thione form. The broad bands at 3424 and 3411 cm\textsuperscript{-1} in L\textsuperscript{1}H substituted complex, respectively, are due to \(\nu(\text{OH})\) of water molecules and hydrogen bonds of the type N–H···O. The \(\nu(\text{NH})\) bands at 3134 and 3049 cm\textsuperscript{-1} are strongly affected on complexation to Pt(II); the presence of only one band at 3236 cm\textsuperscript{-1} indicates NH group participates in bond formation with metal ion [15]. The \(\nu(\text{C}=\text{O})\) and \(\nu(\text{C}=\text{N})\) bands of L\textsuperscript{1}H at 1702 and 1629 cm\textsuperscript{-1}, respectively, are affected on complexation. With respect to the free L\textsuperscript{1}H, the band at 1239 cm\textsuperscript{-1} corresponding to stretching of the most \(\nu(\text{C}=\text{S})\) character shifts to lower wave number in product, which indicate the stronger coordination of the sulphur atom to the Pt(II). The band at 530 cm\textsuperscript{-1} is also assigned to \(\nu(\text{Pt-N})\) bond formation [16]. The spectrum suggests that the final product is an (S, N\textsubscript{3}) coordinated adjacent chelate and the 2-thiouracil behave as a bidentate ligand in the experimental pH [17]. Similarly the presence of moderately strong band at 636 cm\textsuperscript{-1} for brown product of complex (I) and L\textsuperscript{3}H indicates the formation of Pt-O bond [18].

The aqueous solution of \textit{cis}-[Pt\textit{cis}-dach)(OH\textsubscript{2})\textsubscript{2}]\textsuperscript{2+} and L\textsuperscript{1}H were mixed in a 1:1 molar ratio and the mixture was thermo stated at 60 °C for 48 hours and used for ESI-MS measurement. The ESI mass spectrum of the resulting solution is shown in Figure 7.3.
Figure 7.3: ESI-mass spectrum of the product for complex (I) and L¹H at pH 4.0 in aqueous medium and at ionic strength 0.1 mol dm⁻³ NaClO₄.

It is clear from this spectrum that the ion at m/z 436.20 (minor peak) has become the precursor ion species in the mixture solution and this is tentatively attributed to (L¹H⁺ Pt(II) + dach – H⁺)⁺. The peak at 450.25 indicates the formation of the product (L²H⁺ Pt(II) + dach – H⁺)⁺ (Figure 7.4). Similarly the major peak at 408.3070 assigned the (L³H⁺ Pt(II) + dach – H⁺)⁺; the relative abundance of isotope peaks match the expected values, i.e., m/z 407.3076, m/z 409.3076 and m/z 411.3124 (Figure 7.5) [19].
Figure 7.4: ESI-mass spectrum of the product for complex (I) and L₂H at pH 4.0 in aqueous medium and at ionic strength 0.1 mol dm⁻³ NaClO₄.
Figure 7.5: ESI-mass spectrum of the product for complex (I) and L^3H at pH 4.0 in aqueous medium and at ionic strength 0.1 mol dm^-3 NaClO_4.
Figure 7.6: $^1$H NMR spectra of 2-thiouracil and its substituted product
The $^1$H NMR spectrum of $^1$LH exhibits only two doublets, centered at $\delta$ 7.5 and 6.08 ppm, assigned to C₅ and C₆ protons, respectively. The amide protons are not detected in the spectrum and data suggest that the ligand exist in rapid equilibrium. The $^1$H NMR of the platinum complex, the peak of the C₅ and C₆ proton is shifted significantly. This clearly indicates the coordination of the $^1$LH with metal center (Figure 7.6). Thus the structures proposed here for product ion species, deduced from ESI-mass and NMR spectra are consistent with those derived from other experimental methods.

7.3 Physical measurement

All the spectral scanning and kinetic measurements were done in a Shimadzu UV 2450 spectrophotometer attached to a thermoelectric cell temperature controller (model TCC-240A with an accuracy of ± 0.1 °C). IR Spectra (KBr disc, 4000 – 300 cm⁻¹) were measured in Perkin-Elmer FTIR model RX1 Infrared spectrophotometer. ESI-mass spectra were recorded using a micromass Q-Tof micro™ mass spectrometer in +ve ion mode. $^1$H NMR spectrum was recorded using Bruker AVANCE III 400 MHz spectrometer. The pHs of the solutions were adjusted by adding NaOH/HClO₄ and the measurement were carried out with the help of a Sartorius Digital pH meter (model PB11) with an accuracy of ± 0.01 units. Doubly distilled water was used to prepare all the solutions. All other chemicals used were of AR grade. The reactions were carried out at constant ionic strength (0.1 mol dm⁻³ NaClO₄).

7.4 Kinetic measurements

The kinetics of substitution of coordinated water molecule was followed spectrophotometrically by following the change in absorption at suitable wavelength as a function of time. The working wavelengths were determined by recording the spectra of the reaction mixture over the wavelength range between 200 and 500 nm and are given in Figure 7.1. All kinetic experiments were performed under pseudo-first-order conditions. The reactions were initiated by mixing equal volume of complex and ligand solutions (1.5 mL) in the quartz cuvette. The absorption due to ligands was subtracted by using a 1:1 (molar ratio) ligand : water mixture in the reference cell. The concentration of ligand solution was always large enough (at least a tenfold excess) to provide pseudo-first-order conditions. A typical plot of $\ln (A_\infty - A_t)$ (where $A_t$ and $A_\infty$ are absorbances at time (t) and after completion of the reaction) against time (t) (Figure 7.7) is found to be nonlinear; it
is curved at initial stage and subsequently of constant slope indicating that the reaction proceeds via two consecutive steps. From the limiting linear portion of \( \ln (A_{\infty} - A_t) \) versus time \( t \) curve, \( k_{2\text{(obs)}} \) was obtained. The \( k_{1\text{(obs)}} \) values were obtained from the plots of \( \ln \Delta \) versus time \( t \) where time \( t \) is small (Figure 7.8). Origin software was used for computational works. Rate data, represented as an average of duplicate runs, are reproducible within ±4%.

**Figure 7.7:** A typical kinetic plot of \( \ln (A_{\infty} - A_t) \) versus time \( t \). [complex (I)] = \( 2 \times 10^{-4} \) mol dm\(^{-3}\); [L\(^2\)H] = \( 2 \times 10^{-3} \) mol dm\(^{-3}\); pH = 4.0; \( \mu = 0.1 \) mol dm\(^{-3}\) NaClO\(_4\) and Temperature = 35 °C.

**Figure 7.8:** A typical kinetic plot of \( \ln \Delta \) versus time \( t \). [complex (I)] = \( 2.0 \times 10^{-4} \) mol dm\(^{-3}\); [L\(^2\)H] = \( 2.0 \times 10^{-3} \) mol dm\(^{-3}\); pH = 4.0; \( \mu = 0.1 \) mol dm\(^{-3}\) NaClO\(_4\) and Temperature = 35 °C.
Table 7.1: $10^3 k_{1(obs)}$ (s$^{-1}$) values for ligand concentrations at different temperatures.

[complex (I)] = $2.00 \times 10^{-4}$ mol dm$^{-3}$, pH = 4.0, ionic strength = 0.1 mol dm$^{-3}$ NaClO$_4$.

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7.5 Results and discussion

The pK$_a$ values of the ligands L$^1$H, L$^2$H and L$^3$H are 9.78 and 12.7 [20], 12.0 [21] and 8.76 [22], respectively, at 25°C. So at pH 4.0, ligands remain in the neutral form, which participate in the reaction. The pK$_1$ and pK$_2$ (6.25 and 7.80) for cis-(diaqua(cis-1,2-diaminocyclohexane)platinum(II)) has been evaluated by Irving–Rossotti titration technique [23]. Therefore it can be assumed that at pH 4.0 the reactant exists as the diaqua ion. The reactions were involving a two-step consecutive process; the first step is dependent on ligand concentration whereas the second one is independent. In the first step one aqua ligand was replaced from cis - [Pt(cis-dach)(OH$_2$)$_2$]$_2^+$ by LH. The second is a slower step, where another aqua ligand is substituted. This is the ring closure step. The rate constant for such a process can be evaluated by assuming the following scheme:
Where $A$ is the diaqua species ($I$); $B$ is the single substituted intermediate and $C$ is the final product cis-$[\text{Pt}(\text{cis-dach})(L)]^+$. Formation of $C$ from $B$ is predominant after some time has elapsed.

**7.6 Calculation of $k_1$ for $A \rightarrow B$ step**

The rate constant $k_{1\text{(obs)}}$ for the $A \rightarrow B$ step can be evaluated by the method of Weyh and Hamm [24] as discussed in earlier chapter (Section 2.6). $k_{1\text{(obs)}}$ is derived from the slope of $\ln \Delta$ versus time($t$) plot, when time ($t$) is small (Figure 7.8). A similar procedure is applied for all ligands in the concentration range of 0.002 – 0.006 mol dm$^{-3}$ at constant complex ($I$) concentration of 0.0002 mol dm$^{-3}$ at pH 4.0 and at 30, 35, 40, 45 and 50°C respectively. The $k_{1\text{(obs)}}$ values thus obtained are linearly dependent on the studied concentration range. The $k_{1\text{(obs)}}$ values for different ligand concentrations at different temperatures are given in Table 7.1. The ligand concentration dependence of $k_{1\text{(obs)}}$ values can be explained in terms of rapid formation of an outer sphere association complex between the reactant complex ($I$) and the sulfur end of neutral form of L$^1$H in the A to B stage. The rate increases with increase in ligand concentration and reaches a limiting value (Figure 7.9), which is probable due to the completion of the outer sphere association complex formation. Since the metal ion reacts with immediate environment, further change in ligand concentration beyond the saturation point will not affect the reaction rate and a gradual approach towards limiting rate is observed. At this stage the interchange of the ligands from outer sphere to the inner sphere occurs. The following scheme can be proposed:

\[
\begin{align*}
&A \underset{k_1\text{\ anation}}{\xrightarrow{\text{K_E}}} [\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_2]^{2+} \ldots \text{L-LH} \\
&B \underset{k_2\text{\ chelation}}{\xrightarrow{\text{[Pt(cis-dach)(L\cdot\text{L})]^+ + \text{H}_3\text{O}^+}}}
\end{align*}
\]

Where L-LH indicate bidentate form of L$^1$H, L$^2$H and L$^3$H. Based on the above scheme, a rate expression (7.4) can be derived for the $A \rightarrow B$ step:

\[
\frac{d[B]}{dt} = k_1 K_E [\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_2]^{2+}\text{total} \times [\text{L-LH}] / 1 + K_E [\text{L-LH}] \quad (7.1)
\]
or,

\[
\frac{dB}{dt} = k_{1(\text{obs})} [\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_{2}^{2+}]_{\text{total}}
\]

(7.2)

Where \([\text{Pt}(\text{cis-dach})(\text{H}_2\text{O})_{2}^{2+}]_{\text{total}}\) is the total concentration of the complex, \([\text{LH}]\) is the concentration of the bio-active ligands. Hence it can be written;

\[
k_{1(\text{obs})} = k_{1} K_{E} [\text{LH}] / (1 + K_{E} [\text{LH}])
\]

(7.3)

Where \(k_{1}\) is the rate constant for conversion of outer sphere complex to inner sphere complex and \(K_{E}\) is the outer sphere association equilibrium constant. Equation (7.3) can be rearranged:

\[
\frac{1}{k_{1(\text{obs})}} = \frac{1}{k_{1}} + \frac{1}{k_{1} K_{E} [\text{LH}]}
\]

(7.4)

A plot of \(1/k_{1(\text{obs})}\) versus \(1/[\text{LH}]\) should be linear with an intercept of \(1/k_{1}\) and slope \(1/k_{1} K_{E}\). This was found to be so, at all temperature studied (Figure 7.10), the \(k_{1}\) and \(K_{E}\) values obtained from intercept and from slope to intercept ratios are included in Table 7.2.

---

**Figure 7.9:** Plots of \(10^{3} k_{1(\text{obs})} (s^{-1})\) vs. \([\text{L}^{1}\text{H}]\) at different temperatures; A = 30 °C, B = 35 °C, C = 40 °C, D = 45 °C and E = 50 °C; pH 4.0 and ionic strength = 0.10 mol dm\(^{-3}\) NaClO\(_{4}\).
7.7 Calculation of $k_2$ for the B→C step

The B→C step is the ring closure step in which the second donor site of LH binds the metal centre. Due to the steric hindrance, this chelation step is slower and independent of ligand concentration variation. At each temperature, the $k_2$ values were calculated from the limiting linear portion (where time (t) is large) of the $\ln(\frac{A_{\infty}}{A_t})$ versus time (t) curves (Figure 7.7) and are given in Table 7.2. Unlike $k_1$, $k_2$ was found to be independent of ligand concentration at each of the temperature studied.
Table 7.2: $10^3k_1 \ (s^{-1}), \ 10^5k_2 \ (s^{-1})$ and $K_E$ values for different ligands at different temperatures. [complex (1)] $= 2.00 \times 10^{-4} \ \text{mol dm}^{-3}, \ \text{pH} = 4.0$, ionic strength $= 0.1 \ \text{mol dm}^{-3} \ \text{NaClO}_4$.

<table>
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<th>Temperature (± 0.1 °C)</th>
<th>$10^3k_1 \times (s^{-1})$</th>
<th>$10^5k_2 \times (s^{-1})$</th>
<th>$K_E \ (\text{dm}^3\ \text{mol}^{-1})$</th>
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<tr>
<td></td>
<td>50</td>
<td>12.01</td>
<td>7.20</td>
<td>531</td>
</tr>
<tr>
<td>L$^2$H</td>
<td>30</td>
<td>1.47</td>
<td>1.88</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>2.02</td>
<td>2.35</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>2.70</td>
<td>3.24</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>3.38</td>
<td>4.48</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.15</td>
<td>5.52</td>
<td>364</td>
</tr>
<tr>
<td>L$^3$H</td>
<td>30</td>
<td>1.20</td>
<td>1.72</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>1.53</td>
<td>2.33</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>2.01</td>
<td>3.16</td>
<td>249</td>
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<tr>
<td></td>
<td>45</td>
<td>2.64</td>
<td>4.25</td>
<td>277</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3.51</td>
<td>5.33</td>
<td>368</td>
</tr>
</tbody>
</table>

7.8 Effect of pH on reaction rate

The reaction was studied at five different pH values. At a fixed $2.00 \times 10^{-4}$ mol dm$^{-3}$ [complex (1)], $4.00 \times 10^{-3}$ mol dm$^{-3}$ [L$^1$H] and 0.10 mol dm$^{-3} \ \text{NaClO}_4$ ionic strength, the $10^3k_1(\text{obs})$ values were 6.65, 6.66, 6.69, 6.69 and 6.71 and $10^5k_2(\text{obs})$ values were 3.17, 4.02, 5.10, 6.86 and 8.60 at pH 3.0, 3.5, 4.0, 4.5 and 5.0 respectively at 40 °C. The $k_2(\text{obs})$ values were found to increase with increase in pH in the studied pH range. The enhancement in rate may be explained by the acid dissociation equilibria of the ligands and the complex. Within our studied pH range the ligand, 2-Thiouracil remains unchanged so the effects of pH on rate are therefore due to the change in reactive forms of the reacting complex. The complex changes its form, from aqua to hydroxoqua. The
hydroxo species is more reactive due to the well-known labilising effect of the –OH group via its \( \pi \)-bonding ability and strong electromeric effect. The pH dependence of \( k_{2 \text{obs}} \) can also be readily interpreted in terms of the changes with pH in fractional populations of species involved in the respective reactions. Similar trends in rate constants for both the steps were also observed in case of \( \text{L}_2^2 \text{H} \) and \( \text{L}_3^3 \text{H} \). Notwithstanding in the present kinetic runs, the substitution reactions were followed at a constant pH of 4.0 to avoid complication caused by adding an additional parameter of \([\text{H}^+]\) to the rate equation.

7.9 Effect of temperature on reaction rate

The reaction was studied at five different temperatures for different ligand concentrations and the anation rate constants for both \( \text{A} \rightarrow \text{B} \) \((k_1)\) and \( \text{B} \rightarrow \text{C} \) \((k_2)\) steps are given in Table 7.2. The activation parameters calculated from Eyring plots (Figures 7.11 and 7.12) are given in Table 7.3 and compared with those for analogous systems involving the substitution in square planar platinum(II) complexes.

**Table 7.3:** Activation parameters for analogous systems

<table>
<thead>
<tr>
<th>Systems</th>
<th>( \Delta H_1^\circ ) (kJmol(^{-1}))</th>
<th>( \Delta S_1^\circ ) (JK(^{-1})mol(^{-1}))</th>
<th>( \Delta H_2^\circ ) (kJmol(^{-1}))</th>
<th>( \Delta S_2^\circ ) (JK(^{-1})mol(^{-1}))</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-[Pt(cis-dach)((\text{H}_2\text{O}))(_2)](^{2+})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>/Glutathione</td>
<td>32.9 ±1.3</td>
<td>-187.2 ± 4.2</td>
<td>30.5 ± 0.1</td>
<td>-223.1 ± 0.3</td>
<td>[26]</td>
</tr>
<tr>
<td>/diethylthi狄thio-carbamate</td>
<td>66.8 ± 3.7</td>
<td>-81 ± 12</td>
<td>95.1 ± 2.8</td>
<td>-34.4 ± 9.1</td>
<td>[27]</td>
</tr>
<tr>
<td>/DL-Penicillamine</td>
<td>36.1 ± 4.1</td>
<td>-175 ± 12</td>
<td>44.4 ± 1.1</td>
<td>-189 ± 3</td>
<td>[25]</td>
</tr>
<tr>
<td>/(\text{L}_1)\text{H}</td>
<td>14.1 ± 1.0</td>
<td>-239 ± 3</td>
<td>24.9 ± 1.2</td>
<td>-248 ± 4</td>
<td>[**]</td>
</tr>
<tr>
<td>/(\text{L}_2)\text{H}</td>
<td>39.7 ± 1.5</td>
<td>-168 ± 5</td>
<td>43.0 ± 1.5</td>
<td>-194 ± 5</td>
<td>[**]</td>
</tr>
<tr>
<td>/(\text{L}_3)\text{H}</td>
<td>41.1 ± 2.0</td>
<td>-166 ± 6</td>
<td>44.1 ± 0.7</td>
<td>-191 ± 2.1</td>
<td>[**]</td>
</tr>
</tbody>
</table>

** This work

7.10 Mechanism and conclusion

All the ligands exist as neutral molecules at experimental pH 4.0. Thus at the outset a rapid equilibrium is established, which results an outersphere association complex between complex \((I)\) and ligand. Job’s method of continuous variation indicates
1:1 molar ratio and the IR spectra of the solid products suggest that all the ligands behave as a bidentate ligand. Finally $^1$H NMR and ESI-mass spectra provide a qualitative picture of the composition i.e. the ligational sites of the nucleophiles. Thus the mechanism of substitution of aqua ligands in cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) ion can be explained in terms of rapid outer sphere association complex formation, followed by two consecutive steps; the first is dependent on ligand concentration and second is the chelation i.e. ring closure step, which is slower than the first step and independent of ligand concentration (Figure 7.13).

The rate constant ‘$k_1$’ for the ligands 2-thiouracil with (S, N) donor set, 1,2-cyclohexanedionedioxime having (N, N) and acetylacetone having (O, O) donor set, can be rearranged as:

$$2\text{-thiouracil} > 1,2\text{-cyclohexanedionedioxime} > \text{acetylacetone}$$

The sulfur end of 2-thiouracil is a soft donor centre and has a large affinity for the soft Pt centre. The sulphur containing ligands have higher reactivity than ligands containing no sulphur atom. This is due to ability of sulphur atom to function both as $\sigma$-donor and as $\pi$-acceptor. Aside this Pt(II) is soft acid and soft-soft interaction initiates the preference of Pt(II) to sulphur ligands. The cyclisation reaction also shows similar trends. For all ligands the cyclisation process occurs with deprotonation, a phenomenon, which is dependent on the pH and on the nature of the metal ion. At pH 4.0, deprotonation is difficult to occur and the reaction becomes slower. With 1,2-cyclohexanedionedioxime, presence of polar side chain probably helps deprotonation by lowering the energy barrier through solvation. Moreover, owing to better solvation of transition state caused by the presence of hydrophilic site in case of 1,2-cyclohexanedionedioxime, shows higher rate than that of acetylacetone. Therefore Pt(II) has greater affinity for nitrogen centre than that of oxygen for the studied ligands.

The trend in the reactivity of the ligands can also be seen in the activation enthalpies for the reactions involving the Complex (I). The higher the nucleophilicity of the ligand, the smaller the activation enthalpy because of stabilization of the transition state. The enthalpies of activation ($\Delta H_1^\neq$ and $\Delta H_2^\neq$) values and negative values of entropies of activation ($\Delta S_1^\neq$ and $\Delta S_2^\neq$) imply a good degree of ligand participation in the transition state (Table 7.3). The positive enthalpy change for breaking the M-OH$_2$ bond is partially
compensated by the formation of M-L bond in the transition state. The participation of LH in the transition state results in a more compact state and negative $\Delta S^\neq$ value is obtained. Further $\Delta S^\neq_2$ is more negative than $\Delta S^\neq_1$, which suggest that compactness has already been achieved in B and the transformation of B to C is only the replacement of another aqua ligand through chelation.

**Figure 7.11:** Eyring plots of $L^1H$, $L^2H$ and $L^3H$ for $k_1$

**Figure 7.12:** Eyring plots of $L^1H$, $L^2H$ and $L^3H$ for $k_2$
Figure 7.13: Plausible mechanism for the substitution of the aqua ligand from cis-[Pt(cis-dach)(H₂O)₂]²⁺ by L²H.
References


Chapter 8

Summary and conclusion
8.1 Introduction

The present study relates to the kinetic and mechanistic investigations of the interactions of amino acids, (L-asparagine, L-arginine and L-glutamic acid), substituted amino acid (DL-penicillamine), dipeptide (glycyl-L-leucine), nucleotide (adenosine), rescue agent (N,N'-diethylthiourea) and biologically relevant nitrogen, oxygen and sulfur donor nucleophiles (2-thiouracil, 1,2-cyclohexanedionedioxime and acetylace tone) with cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) perchlorate in aqueous medium at constant pH (Table 8.1) and mechanistic aspects of ligand substitution on cis-diaqua(ethylenediamine)platinum(II) perchlorate by N,N'-diethylthiourea in aqueous medium at constant pH. Both the substrates are thus (-N-N-) chelated cis-diaqua complexes of platinum(II). All the anticancer platinum drugs after administration in the body undergo hydrolysis to form aqua derivatives before reacting with any kinds of cell components [1]. For in vitro interactions the diaqua derivatives are therefore more suitable and can be used as a model compound. The coordinated water molecule on Pt(II) centre has been shown to be very labile and can more easily be substituted than a stronger nucleophilic group such as chloride. The amino acids and peptides are produced in body as a result of protein digestion by enzymes and remain absorbed in blood. Chemically amino acids and peptides, which contain different donor centres such as oxygen, nitrogen, sulphur etc. are themselves good ligands. Their interactions with anticancer platinum compounds may serve as a model of metal-protein interactions [2]. Amino acids, which differ in the donor atoms, in principle, should reflect different reactivity with the same substrate. Even amino acids and peptides containing same donor centers may show different reactivity and selectivity.

These nucleophiles were investigated because of their different nucleophilicity, steric hindrance, binding properties and biological relevance. This study is of fundamental importance to unravel the mode of action of this class of compounds and it throws more light on the kinetic and mechanistic behavior of platinum antitumour complexes. The goal is to contribute to the elucidation of the whole mechanism employed by these compounds to reach the biological target. From the present investigation, at least, some idea of comparative reactivity may be made.
8.2 Materials

The substrate complexes, cis-[Pt(cis-dach)Cl\textsubscript{2}] and cis-[Pt(en)Cl\textsubscript{2}] (where dach = 1,2-diaminocyclohexane and en = ethylenediamine) were prepared according to the literature methods [3, 4].

A solution of K\textsubscript{2}PtCl\textsubscript{4}, was mixed with an equimolar amount of diaminocyclohexane (dach) in water and allowed to react at room temperature for 6 to 8 h with constant stirring. The water insoluble cis-dichloro(cis-dach)platinum(II) was collected by filtration and washed successively with water, ethanol and acetone. The reactant complex cis-[Pt(cis-dach)(OH\textsubscript{2})\textsubscript{2}](ClO\textsubscript{4})\textsubscript{2} was prepared from cis-dichloro(cis-dach)platinum(II) by hydrolysis in the presence of two moles equivalent of silver perchlorate. The chloro compound sprinkled over the aqueous solution of silver perchlorate and mixture was kept for 24 hours and then filtered to remove AgCl. The diaqua complex was then characterized spectrophotometrically.

The reactant complex, cis-[Pt(en)(H\textsubscript{2}O)\textsubscript{2}](ClO\textsubscript{4})\textsubscript{2} was prepared following the same procedure and characterised by spectral analysis [4].

The procedures of preparation of the reactant complexes are:

\[
\text{K}_2\text{PtCl}_4 \xrightarrow{\text{en/dach}} [\text{Pt(en/dach)Cl}_2] \xrightarrow{2\text{AgClO}_4} [\text{Pt(en/dach)(H}_2\text{O})_2]\text{ClO}_4\textsubscript{2} + 2\text{AgCl}
\]

All chemicals used were of 98% pure and obtained from Sigma Chemical Co.
Table 8.1: Structures along with the pKₐ values of different ligands used in this work.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Negative logarithm of dissociation constant (25 °C)</th>
<th>Actual form of the ligand during complexation</th>
<th>M : L</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-penicillamine</td>
<td>1.90, 7.92, 10.58</td>
<td>![Image of DL-penicillamine structure]</td>
<td>1 : 1</td>
<td>5</td>
</tr>
<tr>
<td>Glycyl-L-leucine</td>
<td>3.18, 8.14</td>
<td>![Image of Glycyl-L-leucine structure]</td>
<td>1 : 1</td>
<td>5</td>
</tr>
<tr>
<td>Adenosine</td>
<td>3.5, 12.5</td>
<td>![Image of Adenosine structure]</td>
<td>1:1</td>
<td>6</td>
</tr>
<tr>
<td>N,N′-diethylthiourea</td>
<td>2.91</td>
<td>![Image of N,N′-diethylthiourea structure]</td>
<td>1:2</td>
<td>7</td>
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<td>L-asparagine</td>
<td>2.02, 8.80</td>
<td>![Image of L-asparagine structure]</td>
<td>1:1</td>
<td>8</td>
</tr>
<tr>
<td>Compound</td>
<td>pK1</td>
<td>pK2</td>
<td>Ratio</td>
<td>Code</td>
</tr>
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<td>----------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>L-arginine</td>
<td>2.17</td>
<td>9.04</td>
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<td>8</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>2.19</td>
<td>4.25</td>
<td>1:1</td>
<td>8</td>
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<td>2-thiouracil</td>
<td>9.78</td>
<td>12.7</td>
<td>1:1</td>
<td>9</td>
</tr>
<tr>
<td>1,2-cyclohexanedione dioxime</td>
<td>12.0</td>
<td></td>
<td>1:1</td>
<td>10</td>
</tr>
<tr>
<td>Acetylacetone</td>
<td>8.76</td>
<td></td>
<td>1:1</td>
<td>11</td>
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</tbody>
</table>

### 8.3 Kinetic study

The advancement of reaction in each case has been monitored by the absorbance measurement at different intervals of time with a Shimadzu spectrophotometer (UV–2101 PC) equipped with a Shimadzu TB 85 thermobath and also with a Shimadzu UV–Vis spectrophotometer (UV–2450), attached to a thermoelectric cell temperature controller (model TCC-240A with an accuracy of ± 0.1 °C). The absorption due to ligand was eliminated by using 1:1 (v/v) ligand solution : water in the reference cell. Conventional mixing technique was followed and pseudo first order conditions with respect to the complexes were maintained throughout the course of the reaction.

### 8.4 General discussion

The pK₁ and pK₂ for cis-[Pt(en)(H₂O)₂](ClO₄)₂ are 5.8 and 7.6 respectively and that for cis-[Pt(cis-dach)(H₂O)₂](ClO₄)₂ have been evaluated by Irving-Rossotti titration technique [12, 13]. It was found to be 6.25 and 7.80 respectively, which refer to the following dissociation processes:

\[
\text{cis-[Pt(cis-dach)(H₂O)₂]}^{2+} \rightarrow \text{cis-[Pt(cis-dach)(H₂O)(OH)]}^{+} + \text{H}^+ 
\]
\[
\text{cis-[Pt(cis-dach)(H}_2\text{O})(\text{OH})]^+ = \text{cis-[Pt(cis-dach)(OH)}_2] + \text{H}^+
\]

It can, therefore, be assumed that the substrate complexes exist essentially as the diaqua ion in the experimental pH. All the reactions were found to be biphasic in nature. For the above systems, the reactions involve a two-step consecutive process; the first step is dependent on ligand concentration whereas the second is independent on ligand concentration (Except N,N'-diethylthiourea; Chapter 5). In the first step one aqua ligand is replaced from \text{cis-[Pt(en/dach)(H}_2\text{O)}_2]^{2+} by the incoming ligand. The second is the slower step, where another aqua ligand is substituted. This is the intramolecular ring closure step (except N,N'-diethylthiourea).

The rate constants for such processes can be evaluated by assuming the following scheme:

\[A \xrightarrow{k_1(\text{obs})} B \xrightarrow{k_2(\text{obs})} C\]

Where A is the diaqua species, B is the mono substituted species and C is the final product. The rate constant \(k_1(\text{obs})\) for the \(A \rightarrow B\) step can be evaluated by the method of Weyh and Hamm using the usual consecutive rate law [14],

\[
(A_\infty - A_t) = a_1 \exp(-k_1(\text{obs}) t) + a_2 \exp(-k_2(\text{obs}) t)
\]

(8.1)

where \(a_1\) and \(a_2\) are constants depending upon the rate constants and the extinction coefficients.

Rearranging the equation (8.1),

\[
(A_\infty - A_t) - a_2 \exp(-k_2(\text{obs}) t) = a_1 \exp(-k_1(\text{obs}) t)
\]

(8.2)

and assuming \(\Delta = a_1 \exp(-k_1(\text{obs}) t)\), then, \(\ln \Delta = \text{constant} - k_1(\text{obs}) t\)

(8.3)

The values of \(\Delta\) are obtained by extrapolation from the difference, \((X - Y)\), in the plot of \(\ln(A_\infty - A_t)\) versus time \((t)\) curve. Then \(k_1(\text{obs})\) is derived from the slope of \(\ln \Delta\) versus time \((t)\) plot when ‘t’ is small. The L-asparagine, L-arginine, L-glutamic acid, adenosine and glycyl-L-leucine interact with the Pt(II) complexes according to the Scheme 8.1:

\[
cis-[Pt(-N-N-)(H}_2\text{O)}_2]^{2+} + \text{GlyleuH} \xrightarrow{k_1, \text{slow}} cis-[Pt(-N-N-)(\text{H}_2\text{O})(\text{GlyleuH})]^{2+} + \text{H}_2\text{O}
\]
Scheme 8.1

Where GlyleuH is the zwitterionic form of glycyl-L-leucine and -N-N- represents 1,2-diaminocyclohexane or ethylenediamine. Based on the above scheme, a rate expression (8.5) can be derived for the A → B step:

$$\frac{dB}{dt} = k_1 [\text{Pt(dach)(H}_2\text{O)}_2]_{\text{total}} [\text{Glyleu}]$$

(8.4)

Where $[\text{Pt(dach)(H}_2\text{O)}_2]_{\text{total}}$ is the concentration of the unreacted complex. Hence it can be written:

$$k_{1(\text{obs})} = k_1 [\text{Glyleu}]$$

(8.5)

where $k_1$ is the second order rate constant for the first aqua ligand substitution. Thus at each of the experimental temperatures, $k_{1(\text{obs})}$ values when plotted against molar concentration values of ligand, produce a straight line passing through the origin. From the slope of the lines the values of the second order rate constants for different temperatures are obtained and they have been shown on Table 8.2 and Table 8.3.

At a particular temperature, the $k_{2(\text{obs})}$ values were obtained from the limiting linear portions of $\ln(A_\infty - A_t)$ versus time (t) curves when ‘t’ is large.

Glycyl-L-leucine interacts with the Pt(II) complexes according to the Scheme 8.1:
Scheme 8.2: Plausible mechanism for the substitution of the aqua ligand from cis-[Pt(cis-dach)(H₂O)₂]²⁺ by glycyl-L-leucine; R = -CH₂CHMe₂.

The scheme 8.2 is applicable for amino acids (L-asparagine, L-arginine and L-glutamic acid), nucleoside (adenosine) and dipeptide (glycyl-L-leucine). The two cis positions of platinum(II) ion are blocked by NH₂ group of dach or en and in view of preference of square planar configuration in its complexes; it is likely that all the above mentioned ligands behave as bidentate ligand in the complex formation.

The sulphur containing substituted amino acid (DL-penicillamine) and other biomolecules (2-thiouracil, 1,2-cyclohexanedionedioxime and acetylacetone) interact according to the Scheme 8.3.

\[
\text{Cis-[Pt(-N-N-)(H₂O)₂]²⁺ + L-LH} \overset{K_E}{\rightleftharpoons} \text{cis-[Pt(-N-N-)(H₂O)(L-L)]⁺ + H₃O⁺} \\
\text{(complex I)} \quad \text{outer sphere association complex}
\]

\[
\text{Cis-[Pt(-N-N-)(H₂O)(L-L)]⁺} \overset{k_{i1}, \text{ slow}}{\rightarrow} \text{cis-[Pt(-N-N-)(H₂O)(L-L)]⁺ + H₃O⁺} \\
\text{(B)}
\]

\[
\text{Cis-} \text{[Pt(-N-N-)(L-L)]⁺} \overset{k_{i2}, \text{ chelation}}{\rightarrow} \text{cis-[Pt(-N-N-)(L-L)]⁺ + H₂O} \\
\text{(B)} \quad \text{(C)}
\]

Scheme 8.3
where L-LH is the zwitterionic form of DL-penicillamine and -N-N- represents 1,2-diaminocyclohexane or ethylenediamine.

Based on the above scheme, the following rate can be derived for the A→B step:

\[
\frac{dB}{dt} = \left( k_1 K_E [\text{Pt(-N-N-)-(H}_2\text{O)}^2+_{\text{total}}][L-LH] \right) / (1 + K_E [L-LH])
\]

or,

\[
\frac{dB}{dt} = k_1(\text{obs}) [\text{Pt(-N-N-)-(H}_2\text{O)}^2+_{\text{total}}]
\]

(8.6)

where \([\text{Pt(-N-N-)-(H}_2\text{O)}^2+_{\text{total}}]\) is the concentration of the unreacted complex.

Hence it can be written,

\[
k_1(\text{obs}) = k_1 K_E [L-LH]/(1+ K_E [L-LH])
\]

(8.7)

where \(k_1\) is the rate constant for conversion of outersphere complex to the innersphere complex and \(K_E\) is the outersphere association equilibrium constant. The above equation can be rearranged as:

\[
1/k_1(\text{obs}) = 1/k_1 + 1/k_1 K_E [L-LH]
\]

(8.8)

Thus a plot of 1/ \(k_1(\text{obs})\) versus 1/ [DL-penicillamine] should be linear with an intercept of 1/ \(k_1\) and slope 1/ \(k_1K_E\). The \(k_1\) and \(K_E\) values are obtained from the intercept and from slope to intercept ratio of the plot.
Scheme 8.4: Plausible mechanism for the substitution of the aqua ligand from \( \text{cis-[Pt(cis-dach)(H}_2\text{O)}_2]^{2+} \) by DL-penicillamine.

The scheme 8.4 is valid for DL-penicillamine, 2-thiouracil, 1,2-cyclohexanedionedioxime and acetylacetone.

The sulphur containing rescue agent, N,N'-diethylthiourea interacts according to the Scheme 8.5.

\[
\begin{align*}
\text{[Pt(dach)(H}_2\text{O)}_2]^{2+} + \text{detu} & \xrightarrow{k_1 \text{ (anation)}} \text{[Pt(dach)(H}_2\text{O)(detu)]^{2+} + H}_2\text{O} & \text{A} \\
\text{[Pt(dach)(H}_2\text{O)(detu)]^{2+} + \text{detu} & \xrightarrow{k_2 \text{ (chelation)}} \text{[Pt(dach)(detu)]}^{2+} + H}_2\text{O} & \text{B}
\end{align*}
\]

\textbf{Scheme 8.5}

Where detu is the neutral form of N,N'-diethylthiourea ligand.

Based on the above scheme and incorporating protonated form of detu a rate expression (8.10) can be derived for the A \( \rightarrow \) B step:

\[
\frac{dB}{dt} = \frac{k_1}{1 + [H^+]/K_1}[\text{cis-Pt(cis-dach)(H}_2\text{O)}_2]^{2+}]_{\text{total}}[\text{N,N'}-\text{diethylthiourea}]_{\text{total}} \quad (8.9)
\]
Where \([\text{cis-Pt(cis-dach)(H}_2\text{O)}_2^{2+}]_{\text{total}}\) is the total concentration of the platinum complex and \([N,N'-\text{diethylthiourea}]_{\text{total}} = [N,N'-\text{diethylthiourea}] (1 + [H^+]/K_1)\); at pH 3.0 where \(K_1\) is the acid dissociation constant of protonated \(N,N'\)-diethylthiourea. Hence it can be written:

\[
k_{1(\text{obs})} = \frac{k_1}{(1 + [H^+]/K_1)} [N,N'-\text{diethylthiourea}]
\]

(8.10)

where \(k_1\) is the second order rate constant for first step aqua ligand substitution (Scheme 8.6). Thus at each of the experimental temperatures, \(k_{1(\text{obs})}\) values when plotted against molar concentration values of ligand, produce a straight line passing through the origin. From the slope of the lines the values of the second order rate constants for different temperatures are obtained and they have been shown on Tables 8.2 and 8.3. At a particular temperature, the \(k_{2(\text{obs})}\) values were obtained from the limiting linear portions of \(\ln(A_{\infty} - A_t)\) versus time \(t\) curve when ‘\(t\)’ is large. The scheme 8.6 is applicable for the sulphur containing ligand \(N,N'\)-diethylthiourea.

**Scheme 8.6:** Plausible mechanism for the substitution of the aqua ligand from \(\text{cis-[Pt(cis-dach)(H}_2\text{O)}_2^{2+}}\) by \(N,N'\)-diethylthiourea.
8.5 Calculation of $k_2$ for the step $B \rightarrow C$

The $B \rightarrow C$ step is intramolecular ring closure and is independent of ligand concentration (except N, N'-diethylthiourea). Due to steric hindrance, these steps were slower. At a particular temperature the slopes of $\ln(A_{\infty} - A_t)$ versus time ($t$) plots at different ligand concentrations were found to be constant in the region where the plots are linear. For different temperatures the $k_2$ values, obtained directly from the limiting slopes, have been shown in Table 8.2. In case of N,N'-diethylthiourea the second order rate constant ($k_2$) for concentration dependent second aqua ligand substitution step follows the rate expression 8.11.

$$k_{2(\text{obs})} = \frac{k_2}{(1 + [H^+]/K_1)} [\text{N,N'-diethylthiourea}] \quad (8.11)$$

The ‘k’ values are the interchange rate constants dependent on the donor ability of the ligands and the $K_E$ values are the equilibrium constants indicative of the formation of outer-sphere association complex (for DL-penicillamine, 2-thiouracil, 1,2-cyclohexanedionedioxime and acetylacetone). As the outer-sphere association complex is mostly stabilized via hydrogen bonding, thus the $K_E$ values may be taken loosely as a measure of hydrogen bonding. However, a complete correlation of rate constants and equilibrium constants for different ligands with the basicity or nucleophilicity of the ligands are not possible since other factors such as structure of ligands, charge, bonding ability of the donor atoms and hydrogen bonding ability in the activated complex play significant role in the stabilization of the transition state.

8.6 Effect of temperature and spectator ligand

Rate constants ($k$) are sensitive to temperature variation. Their temperature dependence can be explained with the help of Eyring equation:

$$\ln k_h/k_B T = -\Delta H^*/RT + \Delta S^*/R \quad (8.9)$$

Here, ‘k’ is rate constant of the reaction, ‘h’ is Planck’s constant, ‘$k_B$’ is Boltzman constant, ‘T’ is temperature in Kelvin scale and ‘R’ is universal gas constant. The values of $\Delta H^*$ and $\Delta S^*$ can be obtained from the slope and intercept of Eyring plot and they have been tabulated on Table 8.4 and Table 8.5 for the two reactants respectively.

Generally for a dissociative activation, $\Delta H^*$, has higher value than for an associative activation because in associative activation the amount of energy required to break M-L bond is partially compensated by the formation M-L bond in the TS. The
entropy of activation, $\Delta S^e$, is the difference in entropy between the transition state and the
ground state of the reactants. In solution where charged particles are involved, solvation
effects often dominate the entropy of activation. For associative activation the transition
state is more compact than the ground state and a large decrease in entropy is observed
i.e. $\Delta S^e$ shows negative value.

A comparison of $pK_a$ values for diaqua form of complexes *viz*, cis-[Pt(en)(H$_2$O)$_2$]$_2$$^{2+}$ and
cis-[Pt(cis-dach)(H$_2$O)$_2$]$_2$$^{2+}$, shows an increase in $pK_1$ values with the increasing $\sigma$ donor
ability of the ligands: NH$_2$CH$_2$R < NH$_2$CHR$_2$. From $pK$ values it can be concluded that
the complex cis-[Pt(en)(H$_2$O)$_2$]$_2$$^{2+}$ contains lower electron density on Pt(II) centre. The
reactions with the Pt(dach) complex were expected to be slower than those with Pt(en), as
the Pt(II) center should be less electrophilic because of the positive inductive effect of the
cyclohexane ring. Thus the activation parameters and the effect of induction indicate an
associative mechanism for the above discussed substitution processes.

### 8.6 Effects of pH on the reaction rate

The effects of pH can be explained by considering the acid-base equilibria of the
substrate complexes and the ligands. The $k_{obs}$ values were found to increase with increase
in pH in the studied pH range. The enhancement in rate may be explained based on acid
dissociation equilibria of the ligands and the complexes. In the studied pH range with
increase in pH, the diaqua complex will be converted into hydroxo aqua complex. The
reactivity of hydroxo aqua complex is usually higher than that of diaqua complex by the
well-known labilising effect of the coordinated hydroxide ion via its p-bonding ability
and strong electromeric effect. At the same time, with the increase in pH, deprotonation
of the ligands also occurs, which is responsible for the enhanced reactivity. In subsequent
kinetic runs, the substitution reactions were followed at a constant pH of 4.0 to avoid
complications from an additional parameter of $[H^+]$ to the rate equation.

### 8.7 Comparison of rate data and conclusion

The rate constant ‘$k_1$’ for the ligands L-asparagine, L-arginine, L-glutamic acid
and glycy-L-leucine, with (O, N) donor atoms; DL-penicillamine and 2-thiouracil with
(S, N) donor atoms; adenosine and 1,2-cyclohexanedionedioxime with (N, N) donor
atoms; N,N’-diethylthiourea with ‘S’ donor atom and acetylacetone with (O, O) donor
atoms, can be rearranged as:
N, N’-diethylthiourea > 2-thiouracil > DL-penicillamine > 1,2-cyclohexanedione dioxime > L-asparagine > acetylacetone > L-arginine > adenosine > L-glutamic acid > glycyll-L-leucine

The sulphur containing ligands have higher reactivity than ligands containing no sulphur atom. This is due to ability of the sulphur atom to function both as σ-donor and as π-acceptor. Pt(II) is a soft acid, soft-soft interaction initiates the preference of Pt(II) to sulphur ligands. The relative order of rate constants (k₁) for the first step of the reaction of sulphur containing ligands at common temperature 35 °C (common to three ligands) is N, N’-diethylthiourea > 2-thiouracil > DL-penicillamine. Among the three sulphur-donor ligands, 2-thiouracil & DL-penicillamine are thiol ligands and N, N’-diethylthiourea is the combination of thiolates (σ-donor) and thioethers (σ-donor, π-acceptor) ligand properties. From a comparison of reaction rates it can be concluded that the variations in size, bulkiness, pKa and solvation of the entering groups are reflected in their properties as nucleophile. The sensitivity of the reaction rates towards the σ-donor properties of the entering ligands is in line with that expected for an associative mode of activation. For example, DL-penicillamine has the lowest reactivity which can be attributed to steric effects involving the two methyl groups on carbon near the sulphur atom. The greater reactivity of 2-thiouracil compare to that of DL-penicillamine is due to anchimeric effect, which reduces the activation energy of the substitution process, arising from hydrogen bonding interactions between substrate complex and suitable groups located in a suitable position of the nucleophile. The anchimeric effect has been reported for d⁸-metal ion systems like Pt(II) and well known for organic reactions. The thiol ligands are good entering groups for the Pt(II) complexes, but thiourea is the better nucleophile. This enhanced reactivity is due to the inductive effect introduced by the two ethyl groups of detu, which over compensate the steric effect. There is no direct correlation to be found between the reactivity and activation enthalpy, ΔH°, and this indicates that the activation entropy is playing an important role in these reactions [15]. This difference in activation enthalpy is due to the fact that detu is much more solvated than the other nucleophiles studied. The chelation step (except N,N’-diethylthiourea) also shows similar trend. For N,N’-diethylthiourea ligand the second order rate constant k₂ for bis complex formation (bimolecular process) it has been found that the second step is slower than the first step.
This trend agrees with the size and structure of the incoming nucleophile. Once the first entering group has bonded to the platinum centre, the incorporation of the second will be more difficult.

The rate constant ‘$k_1$’ for the ligands L-asparagine, L-glutamic acid, L-arginine, and glycyl-L-leucine all have (O, N) donor set, can be rearranged as:

$$\text{glycyl-L-leucine} < \text{L-glutamic acid} < \text{L-arginine} < \text{L-asparagine}$$

The first step is related with the deprotonation of $-\text{COOH}$ group. So increase in rate from L-glutamic acid to L-asparagine via L-arginine is associated with the pKa values of the ligands. The slow rate for the first step of glycyl-L-leucine is due to its greater steric hindrance. Better solvation of the activated complexes for ligands L-glutamic acid, L-arginine and L-asparagine are also responsible for higher value of the rate constant $k_1$.

The chelation step also shows similar trend. Chelation step is accompanied by deprotonation of the $\alpha$-$\text{NH}_3^+$ of the amino acids. So rate constant values for the second step are in accordance with pKa of $\alpha$-$\text{NH}_3^+$ group. For glycyl-L-leucine the ring closure step involves binding of amide group of the peptide linkage to the metal centre through ‘N’. Due to steric hindrance this step is slower.

At the same time for glycyl-L-leucine the cyclisation process occurs with amide deprotonation, a phenomenon, which is dependent on the pH and on the nature of the metal ion. It has been observed that amide deprotonation of dipeptides occurs around physiological pH range. So at pH 4.0, amide deprotonation is difficult to occur for glycyl-L-leucine and the reaction becomes slower.

The order of reactivity of the ligands 1,2-cyclohexanedionedioxime, acetylacetone and adenosine follow the sequence:

$$1,2\text{-cyclohexanedionedioxime} > \text{acetylacetone} > \text{adenosine}$$

With 1,2-cyclohexanedionedioxime, the presence of polar side chain probably helps deprotonation by lowering the energy barrier through solvation. Moreover, owing to better solvation of transition state caused by the presence of hydrophilic site in case of 1,2-cyclohexanedionedioxime, shows higher rate than that of acetylacetone, So Pt(II) has greater affinity for nitrogen centre than that of oxygen for the aforementioned two ligands. Due to steric hindrance adenosine shows comparatively lower rate of substitution.
The trend in the reactivity of the complexes \( \text{cis-}[\text{Pt(cis-dach)}(\text{H}_2\text{O})_2] \) and \( \text{cis-}[\text{Pt(en)}(\text{H}_2\text{O})_2] \) can be arranged from the activation enthalpies for the reactions involving the nucleophile detu, the reactivity order of complexes observed are: \( \text{Pt(dach)} < \text{Pt(en)} \).

The \( \text{cis-}[\text{Pt(cis-dach)}(\text{H}_2\text{O})_2] \) complex is the sterically most crowded one, and the reactions are found to be slower than those with \( \text{cis-}[\text{Pt(en)}(\text{H}_2\text{O})_2] \). Aside this, higher the electrophilicity of the metal center, the smaller the activation enthalpy because of stabilization of the transition state. The reactions with \( \text{cis-}[\text{Pt(cis-dach)}(\text{H}_2\text{O})_2] \) were expected to be slower than those with \( \text{cis-}[\text{Pt(en)}(\text{H}_2\text{O})_2] \), as the Pt(II) center should be less electrophilic because of the positive inductive effect of the cyclohexane ring. These findings are also reflected in the pKa values of the coordinated water molecules.

8.8 Outcome of the present work

◆ Such types of kinetic studies in aqueous medium are very promising in the biological field.
◆ These compounds may be used for pharmacokinetic studies.
◆ Mode of action of possible anticancer drugs and their interactions with biologically important molecules is drawn in a simplified way.
◆ Reactivity pattern of different Pt(II) complexes are compared and a general conclusion is drawn depending on spectator ligands, maintaining the leaving group same (here \( \text{H}_2\text{O} \) molecule).
References
Table 8.2: Rate constants of various steps of substitution at different temperature with \( \text{cis-}[\text{Pt(cis-dach)}(\text{H}_2\text{O})_2]\)^{2+} ion with different ligands at different ligand concentrations and at different temperatures at constant ionic strength, [substrate complex] = 2.0 \times 10^{-4} \text{ mol dm}^{-3}.

<table>
<thead>
<tr>
<th>Rate constant</th>
<th>Ligand</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^3 \times k_1 \text{ (s}^{-1}))</td>
<td>DL-Penicillamine</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
<tr>
<td>(K_E \text{ (dm}^3\text{ mol}^{-1}))</td>
<td>DL-Penicillamine</td>
<td>117 127 167 227 297</td>
</tr>
<tr>
<td>(10^3 \times k_1 \text{ (s}^{-1}))</td>
<td>2-Thiouracil</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
<tr>
<td>(K_E \text{ (dm}^3\text{ mol}^{-1}))</td>
<td>2-Thiouracil</td>
<td>235 355 447 508 531</td>
</tr>
<tr>
<td>(10^3 \times k_1 \text{ (s}^{-1}))</td>
<td>1,2-Cyclohexanedionedioxime</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
<tr>
<td>(K_E \text{ (dm}^3\text{ mol}^{-1}))</td>
<td>1,2-Cyclohexanedionedioxime</td>
<td>171 179 195 258 364</td>
</tr>
<tr>
<td>(10^3 \times k_1 \text{ (s}^{-1}))</td>
<td>Acetylacetone</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
<tr>
<td>(K_E \text{ (dm}^3\text{ mol}^{-1}))</td>
<td>Acetylacetone</td>
<td>188 214 249 277 368</td>
</tr>
<tr>
<td>(k_1 \text{(dm}^3\text{ mol}^{-1}\text{s}^{-1}))</td>
<td>Adenosine</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
<tr>
<td>(k_1 \text{(dm}^3\text{ mol}^{-1}\text{s}^{-1}))</td>
<td>Glycyl-L-leucine</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
<tr>
<td>(k_1 \text{(dm}^3\text{ mol}^{-1}\text{s}^{-1}))</td>
<td>N(_2)N'-diethylthiourea</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
<tr>
<td>(k_1 \text{(dm}^3\text{ mol}^{-1}\text{s}^{-1}))</td>
<td>L-Asparagine</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
<tr>
<td>(k_1 \text{(dm}^3\text{ mol}^{-1}\text{s}^{-1}))</td>
<td>L-Arginine</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
<tr>
<td>(k_1 \text{(dm}^3\text{ mol}^{-1}\text{s}^{-1}))</td>
<td>L-Glutamic acid</td>
<td>20.0 22.5 25 30 35 40 45 50 55 60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$10^3 \times k_2$(s$^{-1}$)</th>
<th>Ligand</th>
<th>20.0</th>
<th>22.5</th>
<th>25</th>
<th>30</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^5 \times k_2$(s$^{-1}$)</td>
<td>DL-Penicillamine</td>
<td>2.56</td>
<td>3.37</td>
<td>4.54</td>
<td>6.32</td>
<td>7.77</td>
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<td>$10^5 \times k_2$(s$^{-1}$)</td>
<td>2-Thiouracil</td>
<td>3.64</td>
<td>4.41</td>
<td>5.10</td>
<td>6.13</td>
<td>7.20</td>
</tr>
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<td>$10^5 \times k_2$(s$^{-1}$)</td>
<td>1,2-Cyclohexaney-dionedioxime</td>
<td>1.88</td>
<td>2.35</td>
<td>3.24</td>
<td>4.48</td>
<td>5.52</td>
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<tr>
<td>$10^5 \times k_2$(s$^{-1}$)</td>
<td>Acetylacetone</td>
<td>1.72</td>
<td>2.33</td>
<td>3.16</td>
<td>4.25</td>
<td>5.33</td>
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<tr>
<td>$10^5 \times k_2$(s$^{-1}$)</td>
<td>Adenosine</td>
<td>2.32</td>
<td>3.18</td>
<td>4.29</td>
<td>5.92</td>
<td>7.44</td>
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<tr>
<td>$10^5 \times k_2$(s$^{-1}$)</td>
<td>Glycyl-L-leucine</td>
<td>2.01</td>
<td>2.63</td>
<td>3.68</td>
<td>5.15</td>
<td>7.22</td>
</tr>
<tr>
<td>$10^2 \times k_2$ (dm$^3$mol$^{-1}$s$^{-1}$)</td>
<td>N,N′-diethylthiourea$^*$</td>
<td>0.64</td>
<td>0.83</td>
<td>1.10</td>
<td>1.55</td>
<td>2.64</td>
</tr>
<tr>
<td>$10^5 \times k_2$(s$^{-1}$)</td>
<td>L-Asparagine</td>
<td>3.12</td>
<td>4.16</td>
<td>5.24</td>
<td>6.93</td>
<td>9.10</td>
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<tr>
<td>$10^5 \times k_2$(s$^{-1}$)</td>
<td>L-Arginine</td>
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<td>3.53</td>
<td>4.45</td>
<td>6.01</td>
<td>8.03</td>
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<tr>
<td>$10^3 \times k_2$(s$^{-1}$)</td>
<td>L-Glutamic acid</td>
<td>2.24</td>
<td>3.07</td>
<td>3.94</td>
<td>5.39</td>
<td>7.34</td>
</tr>
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</table>

*substrate complex] = 1.5 \times 10^{-4} \text{ mol dm}^{-3}

**Table 8.3:** Rate constants of various steps of substitution at different temperature with cis-[Pt(en)(H$_2$O)$_2$]$^{2+}$ ion with different ligands at different ligand concentrations and at different temperatures at constant ionic strength, [substrate complex] = 1.5 \times 10^{-4} \text{ mol dm}^{-3}.
Table 8.4: Comparison of thermodynamic parameters of all the ligands studies for \( cis \)-[Pt\((cis\text{-dach})(H_2O)_2]^{2+}\) complex.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>( \Delta H_1^\circ ) (kJmol(^{-1}))</th>
<th>( \Delta S_1^\circ ) (JK(^{-1})mol(^{-1}))</th>
<th>( \Delta H_2^\circ ) (kJmol(^{-1}))</th>
<th>( \Delta S_2^\circ ) (JK(^{-1})mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-Penicillamine</td>
<td>36.1 ± 4.1</td>
<td>-175 ± 12</td>
<td>44.4 ± 1.1</td>
<td>-189 ± 3</td>
</tr>
<tr>
<td>2-Thiouracil</td>
<td>14.1 ± 1</td>
<td>-239 ± 3</td>
<td>24.9 ± 1.2</td>
<td>-248 ± 4</td>
</tr>
<tr>
<td>1,2-Cyclohexane-dionedioxide</td>
<td>39.7 ± 1.5</td>
<td>-168 ± 5</td>
<td>43.0 ± 1.5</td>
<td>-194 ± 5</td>
</tr>
<tr>
<td>Acetylacetone</td>
<td>41.1 ± 2.0</td>
<td>-166 ± 6</td>
<td>44.1 ± 0.7</td>
<td>-191 ± 2.1</td>
</tr>
<tr>
<td>Adenosine</td>
<td>43.1 ± 1.3</td>
<td>-177 ± 4</td>
<td>47.9 ± 1.8</td>
<td>-181 ± 2.1</td>
</tr>
<tr>
<td>Glycyl-L-leucine</td>
<td>51.9 ± 2.8</td>
<td>-152 ± 8</td>
<td>54.4 ± 1.7</td>
<td>-162 ± 5</td>
</tr>
<tr>
<td>N,N'-diethylthiourea</td>
<td>62.8 ± 2.1</td>
<td>-26 ± 7</td>
<td>66.2 ± 3.4</td>
<td>-61 ± 11</td>
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<tr>
<td>L-Asparagine</td>
<td>40.7 ± 1.8</td>
<td>-124 ± 6</td>
<td>44.2 ± 0.3</td>
<td>-191 ± 0.9</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>41.5 ± 1.4</td>
<td>-123 ± 4</td>
<td>46.3 ± 0.2</td>
<td>-186 ± 0.6</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>43.3 ± 1.8</td>
<td>-121 ± 6</td>
<td>49.2 ± 0.1</td>
<td>-177 ± 0.3</td>
</tr>
</tbody>
</table>

Table 8.5: Comparison of thermodynamic parameters of all the ligands studies for \( cis \)-[Pt(en)(H_2O)_2]^{2+} complex.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>( \Delta H_1^\circ ) (kJmol(^{-1}))</th>
<th>( \Delta S_1^\circ ) (JK(^{-1})mol(^{-1}))</th>
<th>( \Delta H_2^\circ ) (kJmol(^{-1}))</th>
<th>( \Delta S_2^\circ ) (JK(^{-1})mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>N,N'-diethylthiourea</td>
<td>60.4 ± 3.8</td>
<td>-33 ± 13</td>
<td>62.7 ± 4.5</td>
<td>-71 ± 15</td>
</tr>
</tbody>
</table>
List of Publications


5. Mechanistic and kinetic investigation on the interaction of model platinum(II) complex with ligand of biological significance in reference to antitumour activity (accepted in ‘Synth. & React. in Inorg. Metal-Organ. and Nano-Metal Chem.’).

6. Kinetics and mechanism of some bioactive ligands with cis-diaqua(cis-1,2-diaminocyclohexane)platinum(II) perchlorate in aqueous medium (accepted in *J. Chem. Sci.*).


9. Kinetic studies on substitution of cis-diaqua-chloro-tris-(dimethylsulphoxide)-


12. Mechanistic aspects of ligand substitution on

\[(\text{H}_2\text{O})_2\text{RuORu(tap)}_2(\text{H}_2\text{O})]^2+ \{\text{tap}=2-(m\text{-tolylazo})\text{pyridine}\} \text{ ion by three glycine-containing dipeptides in aqueous medium at physiological pH - A. Mandal, S. Mondal, P. Karmakar, B. K. Bera, S. Mallick and A. K. Ghosh, *J. Chem. Sci.*, **124**, 587 (2012).\]