Mechanistic aspects of the ligand substitution reactions on *cis*-[Pt(1,3-diaminopropane) $(H_2O)_2]^{2+}$ ion with important bio-molecule in aqueous medium

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Abstract

The spectrophotometric study of the interactions of the title complex with DL-penicillamine, a bio-molecule, in aqueous medium as a function of [substrate complex], [ligand], and temperature at constant ionic strength have been performed. The interactions with the studied ligands reveal two phases at pH 4.0: the first is dependent on ligand concentration, while the second is independent. An associative activation mechanism for interaction process is proposed based on the experimental data. The activation parameters and the thermodynamic parameters were computed using the temperature dependences of the outer sphere association, which supports the spontaneous formation of an outer sphere association complex. The product of this reaction has been characterized with the help of Job's method and IR spectroscopic analysis. The bio-active nucleophile's reactivity pattern towards the substrate complex has been discussed, which throws more light on the mechanistic behaviour of platinum(II) antitumor complexes.

Keywords: Mechanism • UV/Vis spectroscopy • Platinum(II) • DL-penicillamine

Introduction

Cis-diaminedichloroplatinum(II), [cis-$(NH_3)_2PtCl_2$], clinically called cisplatin, is one of the most victorious anticancer compound against cancer of the chest, ovaries, bladder, head, and neck but severe toxicities limit its use [1-3]. After the discovery of its activity, thousands of platinum complexes have been synthesized and evaluated for their anticancer activity. Ligand substitution reactions of Pt(II) square-planar complexes have received much interest from various groups over the last two decades [4, 5]. The significance in this field continues successively as demonstrated by the large number of papers appearing yearly. Some of these complexes are of special interest because of their non aqueous chelating leaving groups like Oxaliplatin or Carboplatin.

Interactions of Pt(II) anticancer drugs with sulfur containing bio-relevant ligands have attracted a lot of attention in studies on the biological activity of cisplatin and carboplatin analogues. These compounds usually prefer soft sulfur donors over nitrogen donors [6, 7].

I choose 'S' containing molecule, DL-penicillamine, having three potential donor groups, viz. the free α-amino group, the carboxylate group, and a single reducing group, thiol due to their biological importance [8, 9]. Especially against nephrotoxicity, thiols, were used to prevent or reverse the formation of Pt-S adducts in proteins [10].

The interaction of platinum based drugs with DNA is now widely accepted as the mechanism responsible for their anticancer activity [11-16]. The goal of these studies was to contribute toward the mechanistic understanding of the interaction of the related Pt(II) anticancer drug with DNA and its constituents and smaller molecules, for example, amino acids and inorganic anions. As a part of our interest in the synthesis and reactivity of coordination complexes of Pt(II) with chelating leaving group and with the aim of extending our earlier work, we report here a detailed study on the complex-formation kinetics of $[Pt(dap)(H_2O)_2]^{2+}$ (dap = 1,3-diaminopropane) complex with 'S' containing biologically important molecule such as DL-penicillamine.

Experimental

Materials

K_2PtCl_4 and 1,3-diaminopropane (dap), were purchased from Sigma-Aldrich. DL-penicillamine was purchased from Sisco Research Laboratory. All other reagents used in this research were obtained from commercial sources and used without purification. Solution of the above-mentioned complex and other reagents used for this work were prepared freshly in double-distilled water before use.

Methods

$[Pt(dap)Cl_2]$ were prepared by standard methods and converted into the diaqua complexes by treating them with two equiv. of $AgClO_4$ under light exclusion. The reactant complex *cis*-$[Pt(dap)(OH_2)_2](ClO_4)_2$ ***(1)*** was prepared from *cis*-dichloro(dap)platinum(II) by hydrolysis in the presence of two molar equivalent of silver perchlorate. The chloro compound spread over the aqueous solution of silver perchlorate and mixture was kept in dark for 24 hours and then filtered to remove AgCl.

The products of the reaction between complex (1) and ligand were separately prepared by mixing the reactants at pH 4.0 in different molar ratios, namely 1:1, 1:2, 1:3, 1:4, and 1:5, and thermostating the mixtures at 60°C for 48 h. The absorption spectra of the resulting solutions were recorded and each set were found to exhibit almost identical absorbance at the characteristic wavelengths irrespective of their different molar ratios. The difference in spectra between the product complexes and the substrate complex is shown in Fig. 1. The pH of the solutions was adjusted by adding NaOH/ $HClO_4$ at pH 4.0 so that reactant complex (1) exists as diaqua species in the reaction mixture.

According to Job's method of continuous variation, metal–ligand compositions were found to be 1:1.

Ligand was separately mixed with complex (1) in 1:1 ratio at the experimental pH and six different products were isolated and characterized by IR spectroscopy.

The kinetic measurements of these systems were done according to our previous work [17]. Origin software was used for computational analysis. Rate data, represented as an average of duplicate runs, were reproducible to within ±4%.

Instrumentation

All the spectroscopic scanning and kinetic measurements were done on a Shimadzu UV–VIS spectrophotometer (UV-2450) attached with TCC 240A temperature controller with accuracy (±0.1°C). IR Spectra (KBr disk, 4,000–400 cm^{-1}) were measured with a Shimadzu IR Prestige-21 spectrophotometer. The pH values of the solutions were adjusted by adding NaOH/$HClO_4$, and the measurements were carried using a Sartorius Digital pH meter (model PB11) with an accuracy of ± 0.01 units. Doubly distilled water was used to prepare all solutions. The reactions were carried out at constant ionic strength (0.1 mol dm^{-3} $NaClO_4$).

Results and Discussion

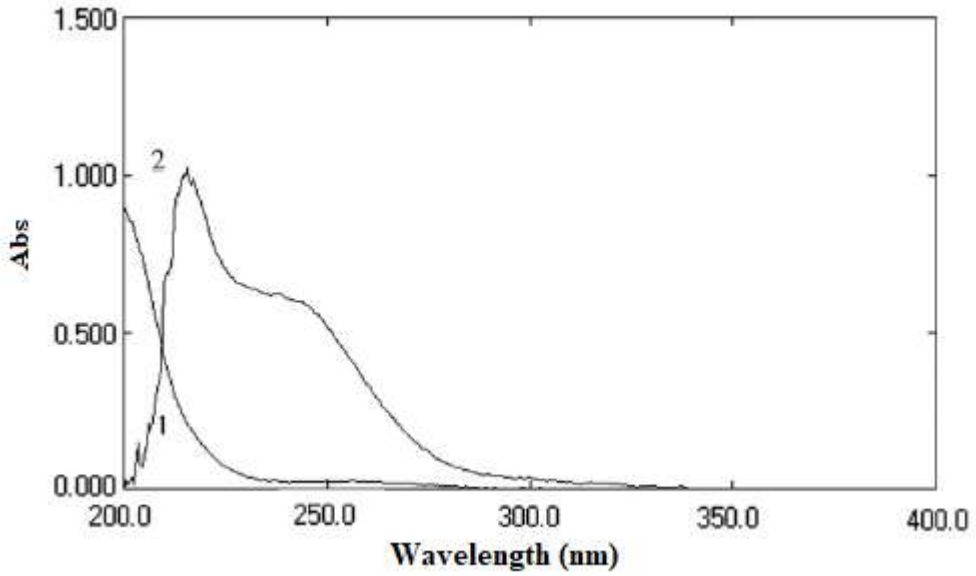


Figure 1: Absorbance differences between [Pt(1,3-diaminopropane)(H_2O)]$^{2+}$ complex (1), DL-penicillamine substituted product (2), [complex 1] = 2× 10^{-4} mol dm^{-3}, [ligand] = 4× 10^{-3} mol dm^{-3}, pH = 4.0, and cell used = 1 cm quartz.

Product Analysis

The electronic transitions observed in the spectra produced by UV-Vis spectroscopy were comparable with what was expected for platinum(II) coordination complexes (Fig. 1). The IR spectra of the compounds were measured in the region 4000-400 cm^{-1} using ATR technology both in solution phase (Fig. 2) and showed characteristic changes when compared to those of the free ligand. The metal–ligand vibrations are expected to occur in a very low frequency range (below 600 cm^{-1}) [18].

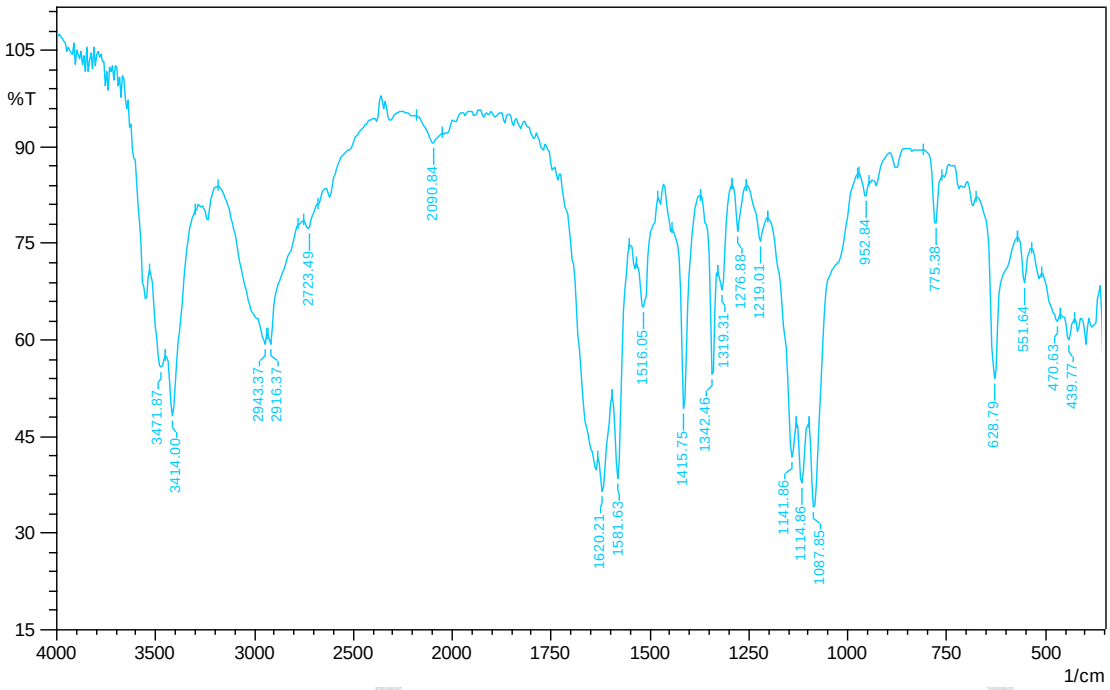


Figure 2: IR spectrum of the DL-penicillamine substituted product of complex (1)

The IR spectra of DL-penicillamine substituted products of complex(1) show, the broad band in the region 3500-3400 cm^{-1} is due to ν_{OH}, which indicates the products are hydrated. The medium and broad stretching vibrations nearly 2916 cm^{-1} correspond to the C-H stretching of CH_3 group. The strong absorption bands at 1620 and 1634 cm^{-1} may be due to overlapping of the νC=N and δNH_2 bending motion coordinated to platinum, indicating COOH is not a ligation site [23]. The bands observed in the region1400-1300 cm^{-1} due to C-N and C-S stretching frequencies shifted in higher frequency than ligand (1140-1080 cm^{-1}). The bands at 551 and 628 cm^{-1} are also assigned to ν(metal–N) bond formation for complex (1) respectively. The spectrum suggests that the final product is an (S, N) coordinated adjacent chelate and the DL-penicillamine behave as a bidentate ligand in the experimental pH.

Kinetic studies

The three dissociation constants pK_1 (-COOH), pK_2 (-SH) and pK_3 ($-NH_3^+$) of DL-penicillamine are 1.90, 7.88 and 10.58 at 25 ^oC respectively [24]. From the pK_a values of the ligands it can be concluded that at pH 4.0, these S containing ligands remain in zwitterionic form, which take part in the reaction. The reactions between complex (1) and ligand involve a two step consecutive route; we put forward that in the first step chelating oxalate ring is opened by the incoming ligand. The second step is slower, where chelate ring closure is occurred. 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, and C is the final product. Formation of C from B is predominant after some time has elapsed.

Calculation of k_1: The rate constant for the first step of the reaction A → B was obtained from the absorbance records using the Origin 6.0 software. The $k_{1(obs)}$ values for different ligand concentrations at different temperatures are given in Table 1.

The following mechanism is proposed:

$$[Pt(dap)(H_2O)_2]^{2+} + \text{L-LH} \underset{}{\overset{K_E}{\rightleftharpoons}} [Pt(dap)(H_2O)_2]^{2+}\ldots\text{L-LH}$$

A Outer sphere association complex

$$[Pt(dap)(H_2O)_2]^{2+}\ldots\text{L-LH} \xrightarrow[\text{anation}]{k_1} [Pt(dap)(H_2O)(\text{L-L})]^{+} + H_3O^{+}$$

B

$$[Pt(dap)(H_2O)(\text{L-L})]^{+} \xrightarrow[\text{chelation}]{k_2} [Pt(dap)(\text{L-L})]^{+} + H_2O$$

B C

Scheme 1

Where L-LH is the zwitterionic form of DL-penicillamine. Based on the above scheme, a rate expression (1) can be derived for the A→B step [19].

$$1/k_{1(obs)} = 1/k_1 + 1/k_1K_E[\text{DL-penicillamine}] \quad (1)$$

A plot of $1/k_{1(obs)}$ *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 (Fig. 3), the k_1 and K_E values obtained from intercept and from slope to intercept ratio, respectively and are included in Table 2.

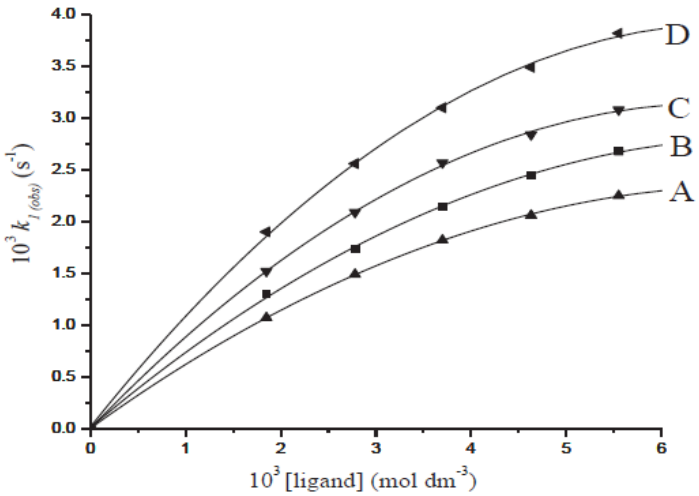


Figure 4: Plots of $k_{1(obs)}$ (s^{-1}) *versus* [DL-penicillamine] at different temperatures. A = 40 °, B = 45 °, C = 50 ° and D = 55 °C; pH 4.0 and ionic strength = 0.10 mol dm^{-3} $NaClO_4$.

Calculation of k_2: The B ⟶ C step is assigned to ring closing for DL-penicillamine. This chelation step is independent of ligand concentration. Slower reaction rate seems to be due to the steric hindrance. At each temperature, the k_2 values were calculated from the limiting linear portion (when t is large) of the $\ln(A_\infty - A_t)$ versus t curves (Fig. 4) and are composed in Table 2. Unlike k_1, k_2 was found to be independent of ligand concentration at each of the temperatures studied.

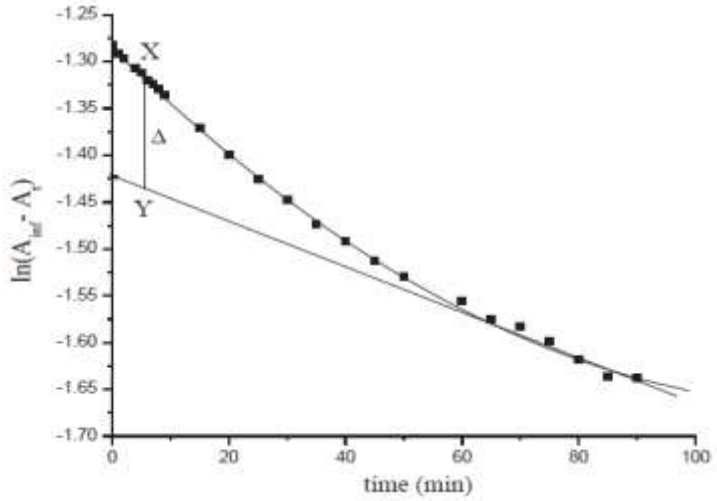


Figure 4: A typical kinetic plot of ln(A_∞ - A_t) *versus* time (t). [complex ***(1)***] = 2×10^{-4} mol dm^{-3}; [DL-Penicillamine] = 4×10^{-3} mol dm^{-3}; pH = 4.0; μ = 0.10 mol dm^{-3} $NaClO_4$ and temperature = 45 °C.

Table 1: $10^3 k_{1(obs)}$ (s^{-1}) values for different concentration of DL-penicillamine at different temperatures. [Complex ***(1)***] = 2×10^{-4} mol dm^{-3}, pH = 4.0, μ = 0.10 mol dm^{-3} $NaClO_4$

Temperature (±0.1° C)	**10^3 [L] (mol dm^{-3})**				
	2.00	**2.50**	**3.00**	**3.50**	**4.00**
40	0.58	0.73	0.87	1.02	1.15
45	0.71	0.89	1.07	1.24	1.42
50	0.90	1.13	1.35	1.57	1.81
55	1.19	1.49	1.78	2.08	2.38

Table 2: k_1 and k_2 values for the substitution reaction

Temperature (±0.1 °C)	**k_1 (mol^{-1} dm^3 s^{-1})**	**$10^5 k_2$ (s^{-1})**
40	0.29	2.63
45	0.35	3.53
50	0.45	4.45
55	0.59	6.01

Table 3: Activation parameters for complex (1) by DL-penicillamine ligand in aqueous medium, at pH 4.0.

Complex	$\Delta H_1^{\neq}$ ($kJ \cdot mol^{-1}$)	$\Delta S_1^{\neq}$ ($J \cdot K^{-1} \cdot mol^{-1}$)	$\Delta H_2^{\neq}$ ($kJ \cdot mol^{-1}$)	$\Delta S_2^{\neq}$ ($J \cdot K^{-1} \cdot mol^{-1}$)
cis-[Pt(1,3-diaminopropane)$(H_2O)_2]^{2+}$	**40.6±3**	**-119±6**	**49.7±3**	**-157±3**

Effect of Temperature on the Reaction Rate

These reactions were monitored at five different temperatures for diverse ligand concentrations and the substitution rate constants for both A B (k_1) and B → C (k_2) steps are arranged in Tables 2. The activation parameters calculated from Eyring plots are tabulated in Table 3.

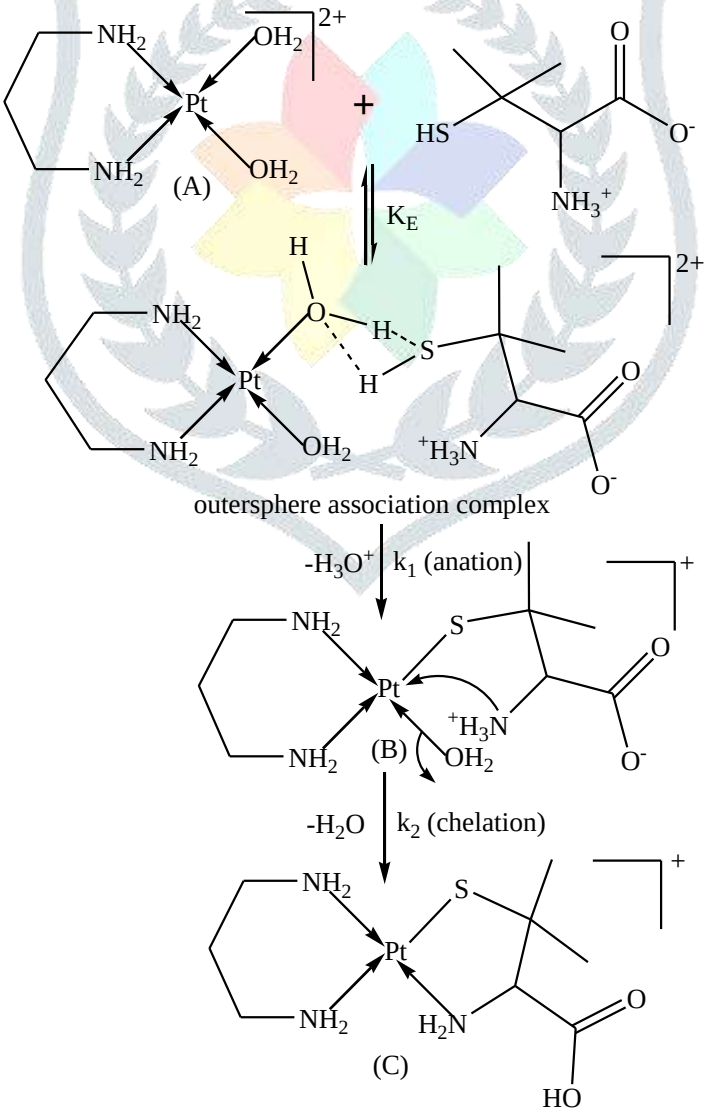
Mechanism and Conclusion

The pharmacokinetic importance of the bicarboxylate ligand emerges mainly from its inertness to hydrolysis under phyosiological conditions and its chelate effect. These complexes react through substitution mechanism excluding the formation of reactive diaqua product.

This study has demonstrated the rate constants of liagnd substitution processes on complex. Biologically important nucleophile, DL-penicillamine, is chosen and rate constants of substitutions, on complex (1) by this nucleophile, were investigated kinetically. The ligand is (S, N) chelator. Pt(II)-amine complexes always exhibits a higher reactivity towards the sulfur donor nucleophiles due to soft-soft interaction between Pt and S atoms.

In this study, DL-penicillamine was found to be the strong nucleophile due to its low pKa value. At first 'S' atom of the DL-penicillamine molecule attacks the metal atom and replace the first aqua molecule, then 'N' atom of amine group completes five member chelate ring and complete dispersal of second aqua molecule occurs (Scheme 2). The first step of this two step process is ligand concentration dependent ring opening step and second step is relatively slow chelation step.

From the negative values of $\Delta S^{\neq}$ it can be concluded that this reaction proceed via associative mechanism.



Scheme 1: Plausible mechanistic pathway

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