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KINETIC STUDIES OF SUBSTITUTION ON *CIS*-DIAQUA-CHLORO-TRIS-(DEIMETHYL SULFOXIDE)-RUTHENIUM(II) COMPLEX WITH GLYCYLGLYCINE IN AQUEOUS MEDIUM

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ABSTRACT

The kinetics of interaction between glycylglycine and *cis*-$[RuCl(Me_2SO)_3(H_2O)_2]^+$ have been studied spectrophotometrically as a function of $[RuCl(Me_2SO)_3(H_2O)_2^+]$, [diglycine] and temperature at a particular pH(5.0), where the substrate complex exists predominantly as a diaqua species(in aqueous solution) and diglycine as the zwitter ion. The reaction has been found to proceed via two distinct consecutive steps i.e., it shows a non-linear dependence on the concentration of diglycine: first process is [ligand] dependent but the second step is [ligand] independent. The rate constants for the processes are: $k_1 \sim 10^{-3}s^{-1}$ and $k_2 \sim 10^{-5}s^{-1}$. The activation parameters were calculated from Eyring plots suggests an associative mechanism for the interaction process. From the temperature dependence of the outer sphere association equilibrium constants, the thermodynamic parameters were also calculated, which gives a negative ΔG^0 value at all temperatures studied, supporting the spontaneous formation of an outer sphere association complex.

INTRODUCTION

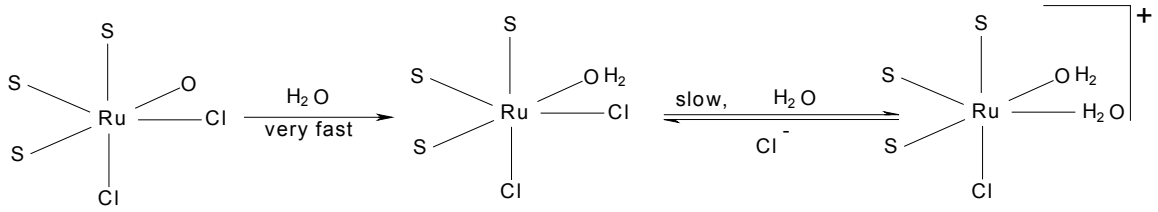
Cisplatin [1] and carboplatin[2] are two well – known drugs for cancer chemotherapy, but certain tumours are resistant to two drugs. Also platinum complexes induce toxic effects such as nephrotoxicity and neurotoxicity. Complexes of other 4d and 5d metal ions, especially ruthenium, rhodium, iridium and palladium, have been reported to have antibacterial power [3, 4]. Complexes of these metal ions with nucleic acid constituents [2, 5 and 6], di [7] and tri [8] peptides and other bioactive ligands [9-12] were studied. Ruthenium(II) complexes are less toxic than cis platin [13,14]. A number of ruthenium compounds serve as bacterial mutagens which indicate that at least some ruthenium complexes are capable of damaging genetic materials [15-18].The studies on the bioactivities of ruthenium(II/III) complexes are still a developing area.

With this background we planned to study the interaction of *cis*-$[RuCl(Me_2SO)_3(H_2O)_2]^+$ with different bioactive ligands e.g., glycylglycine, glycyl-l-leucine, glycyl-l-valine etc. and with certain nucleosides and nucleotides. In the present work the kinetic and mechanistic details of the interaction of diglycine in the aqueous medium at pH 5.0 was examined. The importance of the work lies in the fact that in the aqueous medium the bonding mode of the substrate complex is very interesting and the low pH was used to avoid the oxidation of Ru(II) to Ru(III)[19].

EXPERIMENTAL

The reactant *cis*-$[Ru(Me_2SO)_4Cl_2]$ was prepared and characterized according to the method reported

by Evans et al[20]. The substrate complex $[RuCl(Me_2SO)_3(H_2O)_2]^+$(1) was prepared *in situ* by dissolving the above reactant complex in the aqueous solution[21].



Scheme 1: ***Chemical behavior of cis-[Ru(Me$_2$SO)$_4$Cl$_2$] in aqueous medium.***

Cis-$[Ru(Me_2SO)_4Cl_2]$ once dissolved in water, immediately releases the O-bonded dimethyl sulfoxide molecule [22]. This step was confirmed by conductivity study [21].

The product(***2***) of the reaction between the substrate complex and diglycine was prepared by mixing different molar ratios of reactants, viz., 1:1, 1:2 and 1:3 at pH 5.0 and thermo stating the mixture at 50ºC for 72h. The absorption spectra of the resultant solutions were recorded using an aqueous ligand solution of appropriate molarities in the reference cell, and it was found that the maximum spectral difference between the product complex and the substrate complex, $[RuCl(Me_2SO)_3(H_2O)_2]^+$(***1***) was observed at 259nm(Fi was checked by Job s method of continuous variation as shown in Fig. 2 and was found to have a 1:1 metal:ligand ratio in the product. The pH was adjusted by adding a very small amount of dilute p-toluene sulphonic acid and NaOH solution so that the concentration of the reaction mixture remains constant. Measurements of pH were carried out with the help of a Sartorius digital pH meter (model PB-11) with an accuracy of ±0.01 units. Doubly distilled water was used to prepare all the solutions. All chemicals used were of AR grade.

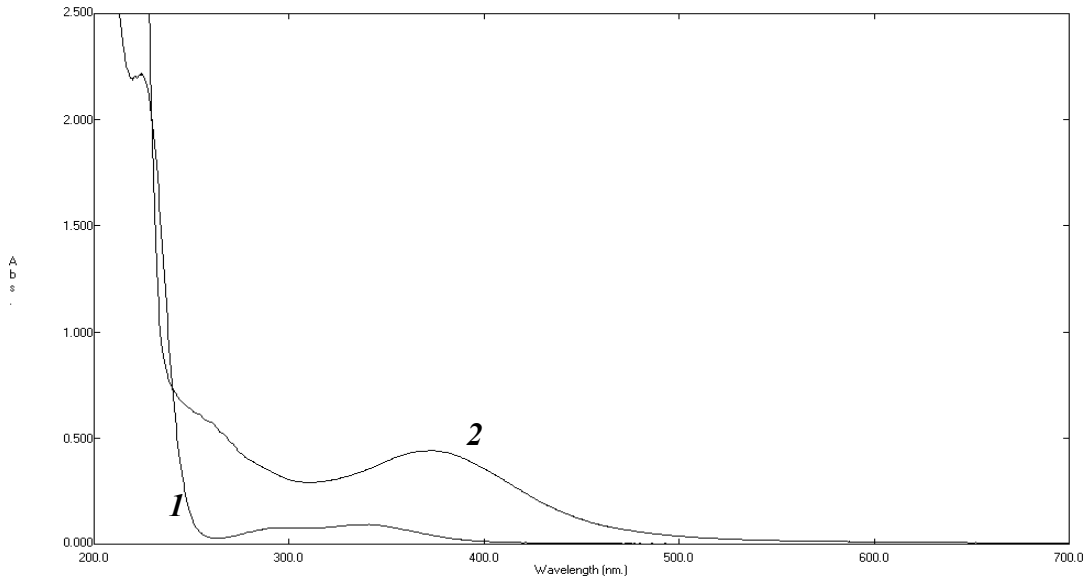


Fig.1. ***Spectral difference between substrate complex and product. (1) [RuCl(Me$_2$SO)$_3$(H$_2$O)$_2^+$] = 1.0×10^{-4} mol dm^{-3}; (2) [RuCl(Me$_2$SO)$_3$(H$_2$O)$_2^+$] = 1.0×10^{-4} mol dm^{-3}, [diglycine] = 2.0×10^{-3} mol dm^{-3}, pH = 5.0***

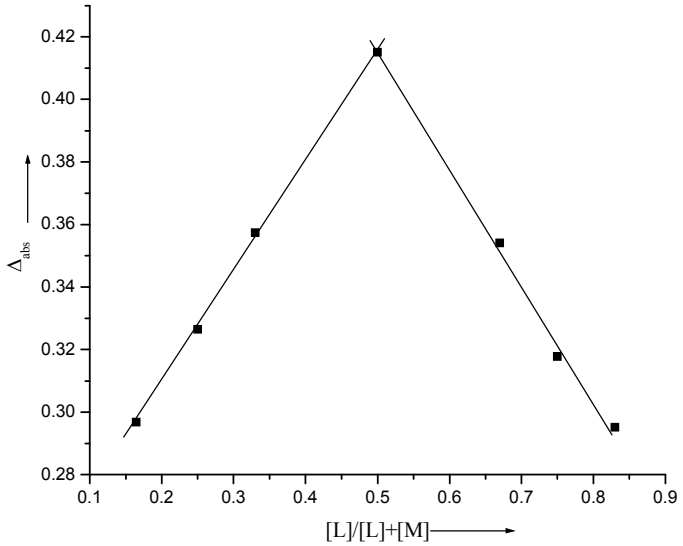


Fig. 2. ***Job's plot: [RuCl(Me$_2$SO)$_3$(H$_2$O)$_2^+$] = 1.0×10^{-4} mol dm^{-3}, [diglycine] = 1.0×10^{-4} mol dm^{-3}, pH = 5.0***

Complex (***1***) and diglycine were mixed in 1:1 molar ratio at pH 5.0 and a yellowish product was obtained. The IR spectra of the yellowish product in the KBr disc show strong band at 3435 cm^{-1} together with medium bands at 1630 and 626 cm^{-1}. The strong band at 3435 cm^{-1} indicates that free carboxylic acid group is present in the product. An intense band of the $\nu(C{=}O)_{amide}$ at 1665 cm^{-1} in the non-coordinated diglycine undergoes a bathochromic ~ 35 cm^{-1} shift in the IR spectra upon complexation. This is probably due to the involvement of the peptide nitrogen (because of the deprotonation that has taken place) in bonding with Ru(II), which lowers the bond order of the $\nu(C{=}O)_{amide}$ group due to resonance stabilization [23]. The absence of stretching frequency in the region 3000 - 3200 cm^{-1} indicates that there is no –N-H bond is present in the product. The 626 cm^{-1} is due to the formation of Ru-N bond in the product [24].

Conductance measurement also helps us to assign the product formation. As with progress of the reaction there is release of $-H^+$ ion (Fig.9) it is expected that conductance of the reacting solution increase with progress of the reaction and it also found experimentally. Due to release of $-H^+$ ion, pH of the resulting solution found to be decreased.

Kinetic studies

Kinetic measurements were carried out on a Shimadzu UV1601PC spectrophotometer attached to a thermoelectric cell temperature controller (model TCC 240A, accuracy ± 0.1). The conventional mixing technique was followed and pseudo-first order conditions were employed throughout. The progress of the reaction was followed by measuring the increase in absorbance at 259 nm, where the spectral difference between the substrate and the product complex is maximum. The $k_{1(obs)}$ and $k_{2(obs)}$ values were calculated graphically (Fig. 3 and 4) using the method of Wyeh and Hamm[25].The rate data represented as an average of duplicate runs are reproducible within ± 4%.

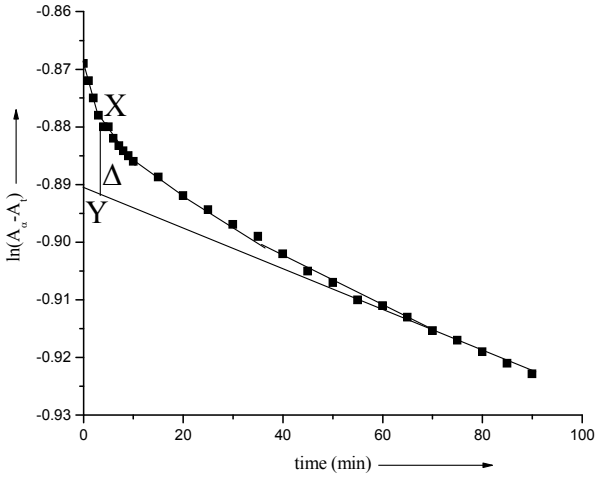


Fig.3. ***A typical plot of ln(A_∞-A_t) versus time t. [$RuCl(Me_2SO)_3(H_2O)_2^+$] = 1.0×10^{-4} mol dm^{-3}, [diglycine] = 2.0×10^{-3} mol dm^{-3}, pH = 5.0, temp. = 50ºC***

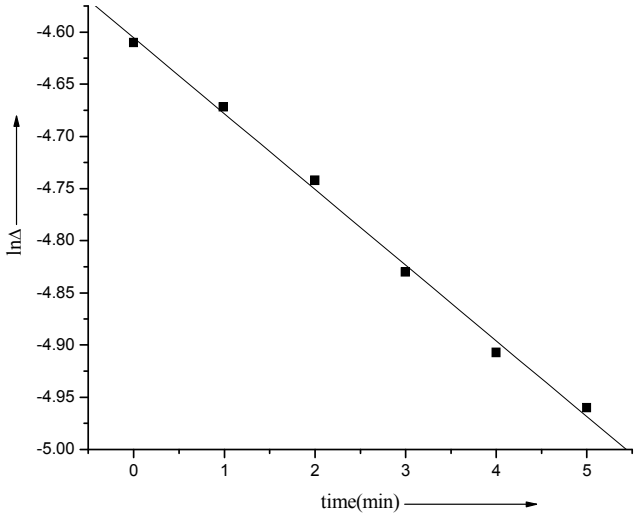


Fig. 4. ***A typical plot of lnΔ versus time t. [$RuCl(Me_2SO)_3(H_2O)_2^+$] = 1.0×10^{-4} mol dm^{-3}, [diglycine] = 2.0×10^{-3} mol dm^{-3}, pH = 5.0, temp. = 50ºC***

RESULTS AND DISCUSSION

Diglycine is the smallest of all dipeptides and contains three separate functional groups; terminal amino group ($–NH_3^+$), terminal carboxylate group ($–COO^-$) and amide group (-CONH–) which is referred to as a peptide linkage. The two dissociation constants are
pK_1(–COOH) 3.21 [26] and pK_2 ($–NH_3^+$) 8.13 [27] at 298 K. Hence at the experimental pH (5.0), diglycine exists as dipolar ion ($H_3N^+–CH_2–CONH–CH_2-COO^-$).

The $\ln(A_\infty - A_t)$ *versus* time, t plot indicates that the reaction is not a single step process, a two step consecutive process may be assumed, both steps are [ligand] dependent.

The rate constant for such process can be evaluated by assuming the following scheme.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

A is the substrate complex, B is the intermediate with ligand diglycine and C is the final product complex $[Ru(Me_2SO)_3(Cl)(L)]$.

Calculation of k_1 value for A→ B step.

The rate constant $k_{1(obs)}$ for A→ B step can be evaluated by the method of Weyh and Hamm using the usual consecutive rate law:

$$(A_\infty - A_t) = a_1 \exp(- k_{1(obs)} t) + a_2 \exp(-k_2 t)$$

or,

$$(A_\infty - A_t) - a_2 \exp(-k_2 t) = a_1 \exp(-k_{1(obs)} t)$$

Where a_1 and a_2 are constants dependent upon the rate constants and extinction coefficient. Values of $[(A_\infty - A_t) - a_2 \exp(- k_2 t)]$ are obtained from X - Y at different time t (fig. 3). So $\ln \Delta$ = constant – $k_{1(obs)}$ t. $k_{1(obs)}$ is derived from the slope of the $\ln\Delta$ *versus* t (where t is small) (Fig. 4). A similar procedure is applied for each ligand concentration in the 1.00×10^{-3} mol dm^{-3} to 5.00×10^{-3} mol dm^{-3} range, at constant [(***I***)] (1.0×10^{-4} mol dm^{-3}) at pH = 5.0 and at different temperatures viz. 35, 40, 45 and 50 ^oC respectively. The $k_{1(obs)}$ values are collected in Table 1.

The rate increases with increase in [ligand] and reaches a limiting value (fig. 5), which is probable due to the completion of the outersphere association complex formation. Since the metal ion reacts with immediate environment, further change in [ligand] 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, i.e., diglycine attacks the Ru(II) atom of the substrate complex and forming intermediate.

Table 1. ***10^3 $k_{1(obs)}$ values for different ligand concentrations at different temperatures.***
[Complex-1] = 1.0×10^{-4} mol dm^{-3}, pH =5.0,

10^3[Ligand] (mol dm^{-3})	Temperatures (°C)			
	35	40	45	50
1.00	0.86	1.14	1.50	1.92
2.00	1.46	1.96	2.60	3.40
3.00	1.98	2.58	3.35	4.18
4.00	2.43	3.13	4.00	4.81
5.00	2.78	3.70	4.40	5.25

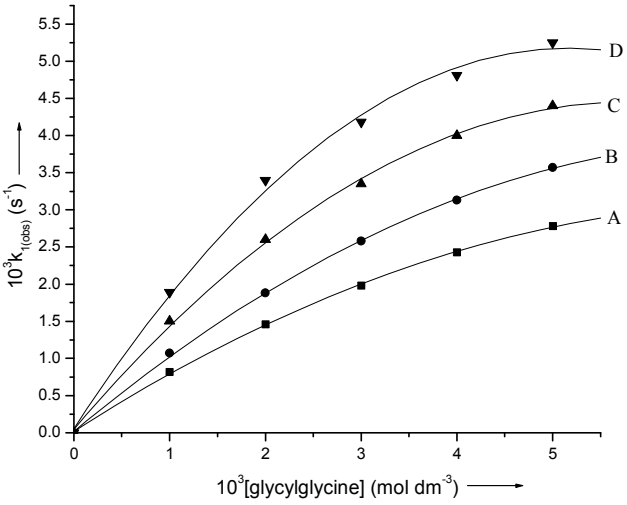


Fig. 5: ***Plot of $k_{1(obs)}$ versus [diglycine] at different temperatures. A = 35, B = 40, C = 45, and D = 50 °C.***

$$A + \text{ligand} \underset{}{\overset{K_{E1}}{\rightleftharpoons}} \underset{\text{Outer sphere association complex.}}{A.\text{ligand}}$$

$$A.\text{ligand} \xrightarrow{k_1} B$$

Scheme-2

Based on scheme -1 a rate expression can be derived for A → B step.

$$d[B]/dt=k_1K_{E1}[B][\text{ligand}]/(1+K_{E1}[\text{ligand}]) \quad (1)$$

$$d[B]/dt=k_{1(obs)}[B]_T \quad (2)$$

T stands for total concentration of Ru(II).Thus it can be written,

$$k_{1(obs)} = k_1 K_{E1} [\text{ligand}] / (1 + K_{E1}[\text{ligand}]) \quad (3)$$

Where k_1 is the rate constant for the formation of intermediate (B) from the substrate complex, *cis*-$[RuCl(Me_2SO)_3(H_2O)_2]^+$ (A). K_{E1} is the outersphere association equilibrium constant.

The equation can be written as

$$1/k_{1(obs)} =1/k_1+1/k_1K_{E1}[\text{ligand}] \quad (4)$$

The plot of $1/ k_{1(obs)}$ *versus* $1/ [\text{ligand}]$ should be linear (fig. 6) with an intercept of $1/ k_1$ and slope $1/ k_1K_{E1}$.

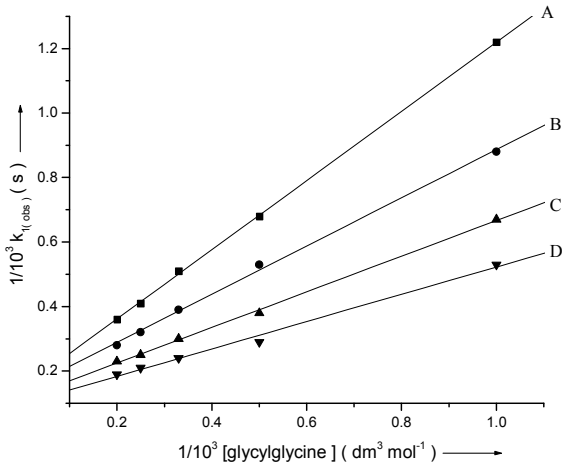


Fig. 6. ***Plot of 1/ $k_{1(obs)}$ versus 1/[diglycine] at different temperatures, A = 35 , B = 40 , C = 45, and D = 50 °C .***

The k_1 and K_E values obtained from the intercept and from slope to intercept ratios are given in Table 2

Table 2: ***$10^3 k_{1(obs)}$ and K_{E1} values at different temperatures***

Temperatures (°C)	10^3 k_1 (s^{-1})	K_{E1} (dm^{-3} mol^{-1} s^{-1})
35	5.89	170
40	7.62	175
45	8.77	206
50	9.76	249

Calculation of k_2 for B → C step:

The B → C step is intramolecular ring closure and is independent of ligand concentration. At a particular temperature the slope of $\ln(A_\infty - A_t)$ *versus* time plot at different ligand concentrations was found to be constant in the region where the plot is linear (Fig. 3). For different temperatures the k_2 values are obtained directly from the limiting slope and the average $10^5 k_2$ values were 4.54, 6.35, 8.72 and 11.03 s^{-1} at 35, 40, 45 and 50 °C respectively.

Effect of change in pH on the reaction rate

The reaction was studied at five different pH values. In the studied pH range it has a great tendency to convert Ru(II) to Ru(III) but the ligand remains unchanged, so it is expected that the reaction rate of the first step will be increased with increase in pH. On the other hand the $k_{2(obs)}$ values are dependent only on the nature of the ligand during chelate formation. So $k_{2(obs)}$ values are independent on pH. Actually it was found true experimentally under our studied pH region (pH 5.0 to 7.0). The $k_{1\,(obs)}$ values are collected in table 3.

Table 3: ***The 10^3 $k_{1(obs)}$ values at different pH values; .***
[$RuCl(Me_2SO)_3(H_2O)^{2+}$] = 1.0×10^{-4} mol dm^{-3}, [diglycine] = 3.0×10^{-3} mol dm^{-3}, Temp. = 50ºC

pH	$10^3k_{1(obs)}$ (s^{-1})
5.0	4.18
5.5	4.82
6.0	5.21
6.5	5.39
7.0	6.18

Effect of temperature on the reaction rate

Four different temperatures with varied ligand concentrations were chosen and the results are listed in Table 3. The activation parameters for the step A → B and B → C are evaluated from the linear Eyring plots (Fig. 7 and 8).

Table 6. ***Activation parameters for [complex- 1] by diglycine in aqueous medium, pH = 5.0***

Ligand	$\Delta H_1^{\neq}$ (kJ mol $^{-1}$)	$\Delta S_1^{\neq}$ (J K^{-1} mol^{-1})	$\Delta H_2^{\neq}$ (k J mol^{-1})	$\Delta S_2^{\neq}$ (J K^{-1} mol^{-1})	ref.
Azide	20.1 ± 3.49	-162 ± 11	35.5 ± 4.2	-105 ± 13	[28]
diglycine	24.7 ± 2.6	-207 ±8	46.8 ± 0.8	-176 ± 2	this work

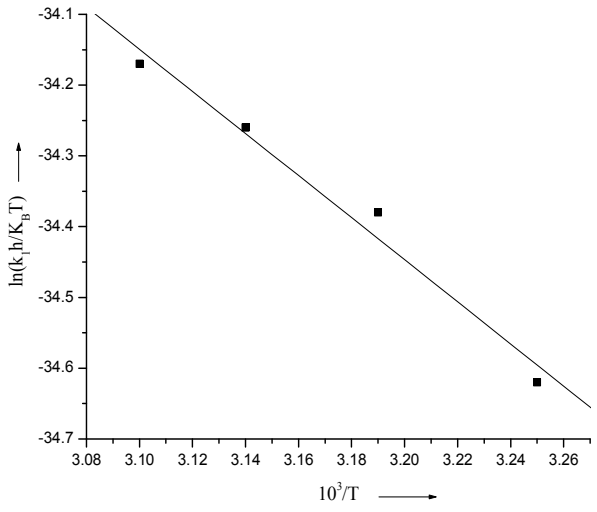


Fig. 7. ***Eyring plot (ln k_1h / k_BT versus 1/T) for the step A → B.***

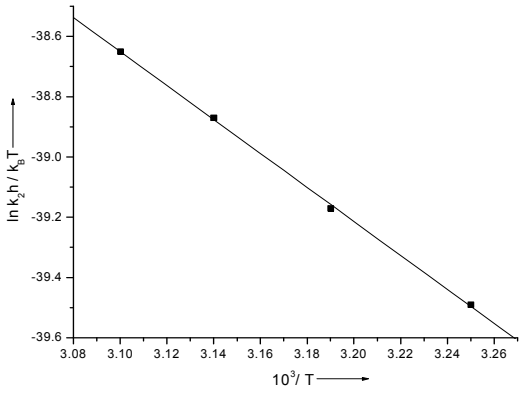


Fig. 8. ***Eyring plot (ln k_2h / k_BT versus 1/ T) for the step B → C.***

The low $\Delta H^{\neq}$ values are in support of the ligand participation in the transition state for both steps. The positive energy required for the bond breaking process is partly compensated for by the negative energy obtained from bond formation in the transition state and, hence, a low value of $\Delta H^{\neq}$ is observed. The highly negative $\Delta S^{\neq}$ values, on the other hand, suggest a more compact transition state than the starting complexes and this is also in support of the assumption of a ligand participated transition state. $\Delta H_2^{\neq}$ is higher than $\Delta H_1^{\neq}$ which is quite expected for the second step which is slower than the first step.

Mechanism and Conclusion

Our results indicates that the first step i.e. the attack by the incoming ligand (diglycine) proceed by an associative interchange (Ia) mechanism. This proposition is supported by the following facts. First, with an increase in ligand concentration saturation in rate is observed. This is possible only when an outer sphere association complex is formed.

Secondly, the low enthalpy of activation and large negative value of entropy of activation strongly suggested the ligand participation in the transition state.

In first step a rapid equilibrium is established, results an outer sphere complex between complex-***1*** and ligand diglycine. The second step is the intramolecular ring closure which is independent on the incoming ligand concentration supported by the value of rate constant (k_2) for this step was found to be actually independent on ligand concentration.

From IR data it is clear that $-NH_2$ group is not participating in bonding. It was also found that after completion of the reaction, the pH of the solution decreases and conductance of the resulting solution increased which might be due to the release of proton from amide –NH group of dipeptide.

From the temperature dependence of the K_{E1}, the thermodynamic parameters are

calculated: $\Delta H_1^0 = 21.0 \pm 5.3$ kJ mol^{-1}, $\Delta S_1^0 = 111 \pm 14$ J K^{-1} mol^{-1} . ΔG^0 value, calculated for the first step at all temperature studied, have a negative magnitude which is once again in favour of the spontaneous formation of an outersphere association complex.

Here in the product four member ring is formed though there is a possibility of formation of six member ring. From the IR study we already had seen that the carboxylate group remains uncoordinated in the product, that is why formation of six member ring was ruled out.

So from IR data, conductance measurement and Job's plot a plausible mechanism may be shown in the following:

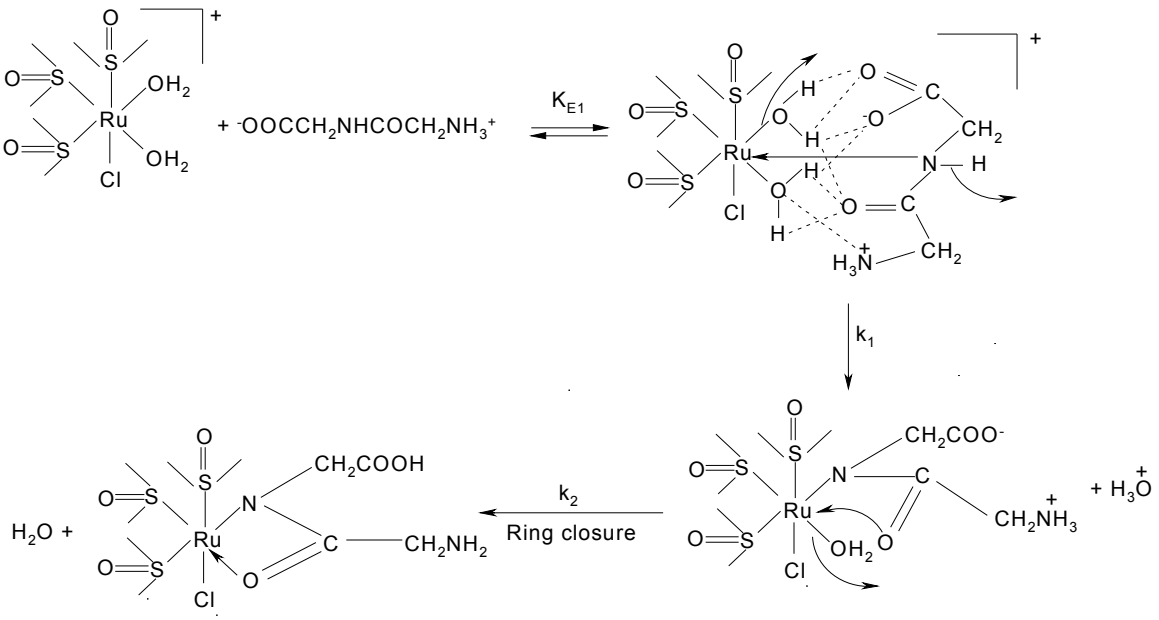


Fig.9: ***Plausible mechanism for the substitution of aqua ligands from cis-[RuCl(Me$_2$SO)$_3$(H$_2$O)$_2$]$^+$***

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