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Reaction of Carbidopa with *cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$ in Aqueous Medium: A Kinetic, Mechanistic and Antiparkinsonian Study of the Product Complex

SUDHANSHU SEKHAR ROUT[1,*], DURGA MADHAB KAR[1], SUDAM CHANDRA SI[1], AJAYA KUMAR PATNAIK[2] and PRAKASH MOHANTY[2]

[1]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar-751030, India
[2]Department of Chemistry, Ravenshaw University, Cuttack-753003, India

*Corresponding author: E-mail: sudhanshurout@soa.ac.in

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For the treatment of Parkinson's disease, the second most common neurodegenerative disorder, requires a combination of levodopa with a peripheral decarboxylase inhibitor, such as carbidopa which provides a symptomatic relief to patients. Reaction of carbidopa with *cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$ has been carried out in aqueous medium over the range $35 \leq t \leq 50$ ºC, $4.0 \leq pH \leq 6.0$, 3.75×10^{-3} mol dm$^{-3} \leq$ [carbidopa] $\leq 9.38 \times 10^{-3}$ mol dm^{-3}, I (KNO_3) = 0.1 mol dm^{-3}. There is outersphere association between *cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$ and conjugate base of carbidopa followed by first chelation. The characterization of the product was performed by using NMR and infrared spectroscopies. The product showed better antiparkinsonian activity than the combination of levodopa and carbidopa.

Keywords: Carbidopa, *cis*-Diaquabis(oxalato)chromate(III), Kinetics, Substitution, Anti-parkinsonian drug.

INTRODUCTION

Parkinson's disease is a progressive neurological syndrome clinically characterized by bradykinesia, involuntary tremors, rigidity and postural instability [1]. This disease caused due to deficiency of an important neurotransmitter like dopamine which is a principal factor for the behavioral changes. Levodopa [L-3,4-dihydroxyphenylalanine] is a precursor of neurotransmitter like dopamine, norepinephrine and epinephrine biosynthesis [2]. In the peripheral circulation, dopamine is formed by rapid decarboxylation of levodopa by aromatic amino acid decarboxylase (AAAD), which resulting in unpleasant side effects and decrease levodopa efficacy. Hence, along with levodopa the AAAD inhibitor, carbidopa [(3,4-dihydroxybenzyl)-2-hydrzinopropionic acid], is routinely co-administered for the treatment of Parkinson diseases. Carbidopa competitively inhibits the extra cerebral decarboxylation of levodopa to dopamine and hence reducing the unpleasant side effects along with improving levodopa efficacy by increasing the proportion of the levodopa dose available for transport across the blood-brain barrier [3-12].

Because of the important biological significance of $Cr^{3+}_{(aq)}$ the substitution reaction of *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$ [13-15] with biomolecules have attracted attention. The kinetics of substitution reactions of $Cr^{3+}_{(aq)}$ with numerous amino acids have been reported [16-29]. In recent times, the reaction between 1,3-propanediamine-*N*,*N*-diacetate-N,N′-di-3-propionate with $Cr(H_2O)_6^{3+}$ has been reported [30]. Generally, the substitution reactions of $Cr^{3+}_{(aq)}$ follows I_a mechanism [31]. The present work pertains to the reaction of *cis*-$[Cr(C_2O_4)_2\text{-}(OH_2)_2]^-$ with carbidopa. The objective of the present study is to know the reaction path and to know whether the product exhibits antiparkinsonian property or not. The substitution reaction of $Cr(C_2O_4)_2(H_2O)_2^-$ with carbidopa follows Ia path because 10^4 k_{an} (50 ºC) = 1.11 s^{-1} and 10^6 k_{ex} (25 ºC) = 2.40 s^{-1}. $\Delta S^{\#}$ = -117 J K^{-1} mol^{-1} also supports ordered transition state and I_a mechanism.

EXPERIMENTAL

Chemicals used in this study were of analytical grade. $K[Cr(C_2O_4)_2(H_2O)_2]\cdot 3H_2O$ was synthesized by the procedure as described earlier [32]. In order to know the purity of

$K[Cr(C_2O_4)_2(H_2O)_2]\cdot 3H_2O$, the percentage of chromium in $K[Cr(C_2O_4)_2(H_2O)_2]\cdot 3H_2O$ was determined by iodometric and AAS technique. Levodopa and carbidopa samples were procured from Dr Reddy's Laboratories, India. A SYSTRONICS digital pH meter 335 was used to maintain the pH of solution, which was equipped with a combination of glass Ag/AgCl/Cl^- (3 mol dm^{-3} NaCl) electrode. For the calibration of pH meter standard buffers of pH 4.0, 7.0 and 9.0 (Merck) were used. The absorbance was recorded by using UV-visible spectrophotometer (JASCO V-630) equipped with 10 mm quartz cuvettes. NMR spectrum of the product complex was recorded with Bruker-Avance III 400 spectrometer and a JASCO FTIR-4100 spectrophotometer was used to record the IR spectra.

Test for acute haloperidol-induced catalepsy: The effect of *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$- carbidopa complex on haloperidol induced catalepsy was studied by a bar test which was carried out as per the method reported by Adedeji *et al.* [33]. Healthy adult wistar rats were used in this study and five equal groups were used in all experiments which consist of six animals in each group. Briefly, catalepsy was induced in rats by injecting haloperidol (1mg/Kg, i.p.). After 60 min of haloperidol injection, five treatment regimens were used: Group I: untreated animals; Group II: vehicle-treated animals (Tween + distilled water); Group III: levodopa (50 mg/kg p.o.); Group IV: combination of levodopa (50 mg/kg p.o.) and carbidopa (25 mg/kg p.o.) and Group V: combination of levodopa (50 mg/kg p.o.) and *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$-carbidopa complex (25 mg/kg p.o.). Bar test was performed at 15, 30, 60, 90 and 120 min following haloperidol injection.

The assessment of catalepsy was recorded as the time duration taken by the rats to maintain an imposed position with both forelimbs extended and rested on a 4 cm high wooden bar (1 cm in diameter). The cataleptic end point occurred when either the fore paws of the animal were removed from the bar or the animal showed the movement of its head in an exploratory manner. A cut-off time of 300 s was taken to avoid unnecessary pain and suffering to the animals during the course of the experiment. The animals were subjected to return to their home cage between the two determinations. All the observations were made in a calm and quiet room at 23-25 ºC for the accuracy of animal response to different treatment.

The scoring technique was followed by the way, the animal maintained the imposed posture for at least 20 s was considered as cataleptic and the time was recorded in seconds.

Kinetics measurements: Ionic strength (I) of the solution was maintained by using potassium nitrate. Thermally equilibrated carbidopa solution at given temperature and desired pH was added to the solution of $K[Cr(C_2O_4)_2(H_2O)_2]\cdot 3H_2O$ which was thermally equilibrated previously. Then, the change in absorbance of the solution mixture was monitored at 413 nm. Throughout these experiments pseudo-first-order conditions were maintained by using large excess of carbidopa. [Cr(III) complex]:[carbidopa] were 1:5, 1:6.75, 1:7.5,1:10 and 1:12.5. Due to low solubility of carbidopa in water the ratio of [Cr(III) complex]/[carbidopa] couldn't be increased further. The pseudo-first order rate constant (k_{obs}) was derived from the slope of $\ln(A_\infty - A_t)$ *versus* t plots;

$$\ln(A_\infty - A_t) = \ln(A_\infty - A_0) - k_{obs}t$$

where A_0, A_t and A_∞ represents the absorbances at the beginning, at time t and at infinity time, respectively. The reaction was studied up to 5 half lives and the rate constants were reproducible within ± 5%. The correlation coefficients were 0.98 in most of the cases.

RESULTS AND DISCUSSION

Stoichiometry and reaction products: Carbidopa was added to the acidic solution of $K[Cr(C_2O_4)_2(H_2O)_2]\cdot 3H_2O$ and formation of product was investigated by UV-visible spectral analysis. It was observed that the absorbance was increased in the visible region of spectrum (Fig. 1). The λ_{max} shifted from 378 to 413 nm (red shift) indicated the formation of Cr(III)-carbidopa complex. Further, the formation of a 1:1 Cr(III)-carbidopa complex was confirmed by Job's plot. The tentative structure of the product complex was further supported by FTIR and NMR spctral analysis and is given in Fig. 2.

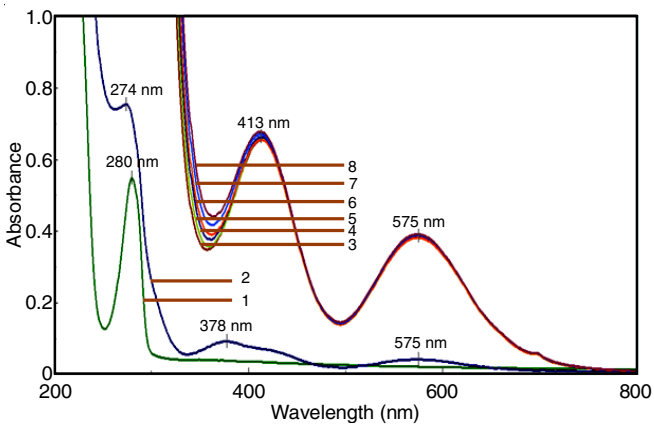


Fig. 1. An overlay scanning plot of Cr(III) + carbidopa at different time intervals. Plot 1. [carbidopa] = 1×10^{-3} mol dm^{-3} Plot 2. $[Cr(III)_T] = 1 \times 10^{-3}$ mol dm^{-3} Plot 3. [carbidopa] = 3.75×10^{-3} mol dm^{-3}, $[Cr(III)_T] = 7.5 \times 10^{-4}$mol dm^{-3}, [carbidopa]/$[Cr(III)_T]$ = 1:5, I = 0.1 mol dm^{-3}, pH = 5.3, 10 min after mixing. Plot 4, 5, 6, 7, 8 are the plots of the mixture after 20, 30, 40, 50 and 60 min after mixture

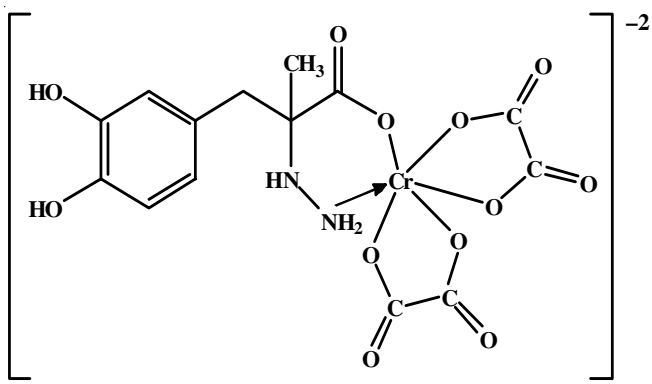
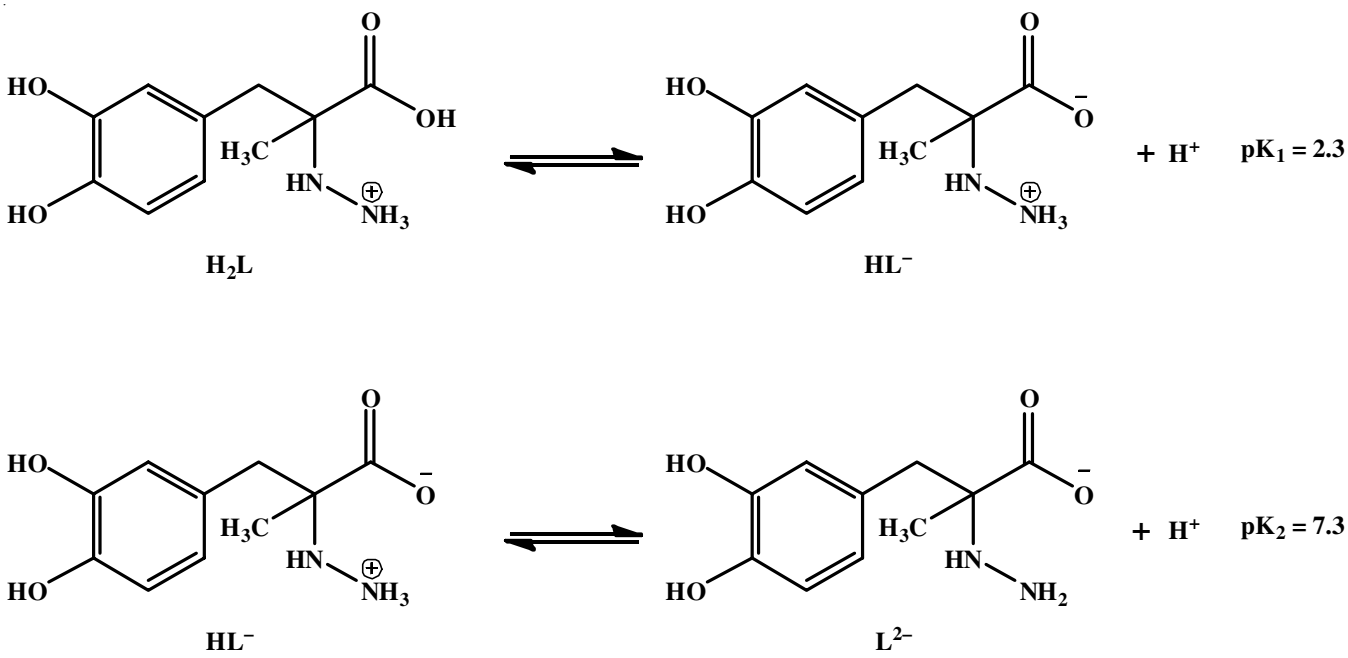


Fig. 2. Tentative structure of the product complex

Effect of variables on the reaction rate: The reaction was studied over a pH range from 4.0 to 6.0 where I = 0.1, [carbidopa] = 9.38×10^{-3} and [*cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$] = 7.5×10^{-4} mol dm^{-3}. The k_{obs} values increased with decrease in $[H^+]$.



Scheme-I: Proton equlibria of carbidopa

It was necessary to consider the deprotonation equilibria of carbidopa to explain the $[H^+]$ dependence on the reaction rate which is shown in **Scheme-I**.

In this substitution reaction, when pH was varied over a range from 4.0 to 6.0, $[HL^-]$ was likely to be the most active species. The pK_1 and pK_2 values [34] of carbidopa at 25 ºC were 2.3 and 7.3, respectively. As per reported data, the hydrolysis constant K_H for *cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$ is 1.94×10^{-8} at 25 ºC [35]; due to this, the concentration of *cis*-$[Cr(C_2O_4)_2(H_2O)(OH)]^{2-}$ will be negligibly small.

Effect of variation of [carbidopa]: The $[carbidopa]_T$ dependence on rate of reaction was studied at 50 ºC keeping the $[H^+]$ and $[cis\text{-}[Cr(C_2O_4)_2(OH_2)_2]^-]_T$ fixed and varying $10^3 \times [carbidopa]_T$ from 3.75 to 9.38 mol dm^{-3}. It was observed that k_{obs} increases in a non-linear fashion with increase in $[carbidopa]_T$, which indicates the formation of an outer sphere complex between *cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$ and carbidopa (Fig. 3). The order of the reaction with respect to $[carbidopa]_T$ was fractional.

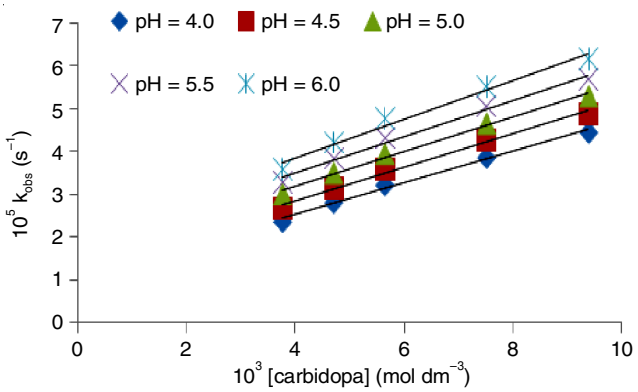


Fig. 3. Plot of 10^5 k_{obs} (s^{-1}) *versus* 10^3 $[carbidopa]_T$ (mol dm^{-3}) at different pH $[cis\text{-}[Cr(C_2O_4)_2(H_2O)_2]^-]_T = 7.5 \times 10^{-4}$, I = 0.1 mol/$dm^{-3}$ (KNO_3), t = 50 °C, 4.0 ≤ pH ≤ 6.0

Effect of variation of [*cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$]: At pH = 4.0, ionic strength = 0.1 mol dm^{-3}, t = 50 ºC and $[carbidopa]_T = 9.38 \times 10^{-3}$ mol dm^{-3}, the 10^4 $[cis\text{-}[Cr(C_2O_4)_2(OH_2)_2]^-]_T$ was varied from 7.5 to 9.0 mol dm^{-3} and it was found that the pseudo-first order rate constant k_{obs} remains almost constant *i.e.* $(4.45 \pm 0.1) \times 10^{-5}$ mol dm^{-3}. This indicates first order dependence of $[cis\text{-}[Cr(C_2O_4)_2(OH_2)_2]^-]_T$ on the reaction rate.

The substitution reaction was carried out at four different temperatures *i.e.* 35, 40, 45 and 50 ºC and at five different pH (4.0, 4.5, 5.0, 5.5 and 6.0). It was found that the values of k_{obs} increased by increasing pH and temperature of the reaction mixture. With increase in pH, the $[HL^-]$ will increase. $[HL^-]$ is the zwitter ionic form of carbidopa and a better nucleophile than H_2L in acidic medium. The data are collected in Table-1.

The mechanism of the reaction is described in **Scheme-II**.

Rate law can be derived in the following manner:

$$\text{Rate} = k_{an}[O.S]_e \quad (1)$$

$$K_{os} = \frac{[O.S]_e}{[\text{Cr(III) carbidopa complex}]_e[HL^-]_e} \quad (2)$$

$$[O.S]_e = K_{OS}\,[\text{Cr(III) carbidopa complex}]_e[HL^-]_e \quad (3)$$

$$\text{Rate} = k_{an}K_{OS}[\text{Cr(III) carbidopa complex}]_e[HL^-]_e$$
$$= k_{an}K_{OS}K_1[\text{Cr(III) carbidopa complex}]_e[H_2L]_e/[H^+] \quad (4)$$

$$[\text{Cr(III) complex}]_T = [\text{Cr(III) carbidopa complex}]_e + [O.S] \quad (5)$$
$$= [\text{Cr(III) carbidopa complex}]_e + K_{OS}[\text{Cr(III) carbidopa complex}]_e\,[HL^-]_e \quad (6)$$
$$= [\text{Cr(III) carbidopa complex}].\{1 + K_{OS}[HL^-]_e\}$$

$$[\text{Cr(III) carbidopa complex}]_e = \frac{[\text{Cr(III) carbidopa complex}]_T}{\{1 + K_{OS}[HL^-]_e\}} \quad (7)$$

TABLE-1
SUBSTITUTION RATE CONSTANTS OF CARBIDOPA WITH *cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$ AT 50 °C, [*cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$] = 7.5×10^{-4} and I = 0.1 mol dm^{-3} IN THE TEMPERATURE RANGE 35 to 50 °C

pH	10^3[Carbidopa]$_T$ (mol dm^{-3})	10^5 k_{obs} (s^{-1})			
		35 °C	40 °C	45 °C	50 °C
4.0	3.75	1.03	1.37	1.80	2.36
	4.69	1.21	1.62	2.24	2.81
	5.63	1.34	1.82	2.45	3.27
	7.50	1.55	2.12	2.87	3.86
	9.38	1.70	2.37	3.26	4.45
4.5	3.75	1.10	1.56	2.01	2.67
	4.69	1.35	1.81	2.39	3.15
	5.63	1.52	2.05	2.74	3.64
	7.50	1.68	2.32	3.15	4.26
	9.38	1.86	2.59	3.56	4.88
5.0	3.75	1.32	1.75	2.29	2.99
	4.69	1.48	1.99	2.64	3.49
	5.63	1.60	2.27	3.17	4.01
	7.50	1.86	2.55	3.45	4.65
	9.38	2.01	2.81	3.86	5.29
5.5	3.75	1.51	1.97	2.54	3.27
	4.69	1.64	2.20	2.91	3.84
	5.63	1.85	2.49	3.33	4.41
	7.50	2.05	2.78	3.73	4.98
	9.38	2.16	3.01	4.13	5.63
6.0	3.75	1.68	2.18	2.79	3.57
	4.69	1.80	2.41	3.19	4.20
	5.63	2.02	2.72	3.62	4.80
	7.50	2.21	2.99	3.99	5.31
	9.38	2.32	3.22	4.41	6.01

$$H_2L \overset{K_1}{\rightleftharpoons} HL^- + H^+ \quad (8)$$

$$K_1 = \frac{[HL^-]_e[H^+]}{[H_2L]_e} \quad (9)$$

$$[HL^-]_e = \frac{K_1[H_2L]_e}{[H^+]} \quad (10)$$

Substituting the value of $[HL^-]_e$ from eqn 10 in eqn. 7.

$$[Cr(III)\ complex]_e = \frac{[Cr(III)\ carbidopa\ complex]_T}{1 + K_{OS}K_1[H_2L]_e/[H^+]}$$

$$= \frac{[H^+]\,[Cr(III)\ carbidopa\ complex]_T}{[H^+] + K_{OS}K_1[H_2L]_e} \quad (11)$$

$$[H_2L]_T = [H_2L]_e + [HL^-]_e \quad (12)$$

$$= [H_2L]_e + K_1[H_2L]_e/[H^+] \quad (13)$$

$$= [H_2L]_e\{([H^+]+K_1)/[H^+]\}$$

$$[H_2L]_e = \frac{[H_2L]_T\ [H^+]}{\{[H^+] + K_1\}} \quad (14)$$

Substituting the value of [Cr(III) carbidopa complex]$_e$ and $[H_2L]_e$ in eqn. 4, we get:

$$Rate = \frac{k_{an}K_{OS}K_1[H^+][Cr(III)\ carbidopa\ complex]_T[H_2L]_T}{([H^+] + K_1)\{[H^+] + K_{OS}K_1[H_2L]_T[H^+]/[[H^+] + K_1]\}}$$

$$= \frac{k_{an}K_{OS}K_1[H^+][Cr(III)\ carbidopa\ complex]_T[H_2L]_T}{[H^+]^2 + K_1[H^+] + K_{OS}K_1[H^+][H_2L]_T}$$

$$= \frac{k_{an}K_{OS}K_1[Cr(III)\ carbidopa\ complex]_T\cdot[H_2L]_T}{[H^+] + K_1 + K_{OS}K_1[H_2L]_T} \quad (15)$$

As we know that

$$Rate = k_{obs}[Cr(III)\ carbidopa\ complex]_T \quad (16)$$

By comparing eqns. 15 and 16:

$$k_{obs} = \frac{k_{an}K_{OS}K_1\cdot[H_2L]_T}{\{[H^+] + K_1\} + K_{OS}K_1[H_2L]_T} \quad (17)$$

If we take the reciprocal of eqn. 17:

$$\frac{1}{k_{obs}} = \frac{1}{k_{an}} + \frac{([H^+]+K_1)}{(k_{an}K_{OS}K_1)} \times \frac{1}{[H_2L]_T} \quad (18)$$

O.S. = outersphere complex; K_{OS} = outersphere association constant.

The rate law mentioned in the above mechanism was tested by plotting k_{obs}^{-1} *versus* [carbidopa]$^{-1}_T$ as indicated in Fig. 4. All the plots were linear with $R^2 = 0.98$ (Fig. 4). The value of anation rate constant, k_{an} was calculated by taking the reciprocal of intercept and the outer sphere association constant (K_{os}) was calculated from the slope of the plots. The data are shown in Table-2. It is established that complexation proceeds by a mechanism in which the rate determining step is the change from an outer sphere to an inner sphere complex. The value for k_{an} at 50 ºC was 1.11×10^{-4} s^{-1}. The much higher value of k_{an} as compared to k_{ex} (2.40×10^{-4}) (25 ºC) [36] favours the fact that the anation reaction follows I_a mechanism. Moreover the negative $\Delta S^{\#}$ also supports I_a mechanism for the substitution reaction.

$$cis\text{-}[Cr(C_2O_4)_2(H_2O)_2]^- + HL^- \overset{K_{OS}}{\rightleftharpoons} cis\text{-}[Cr(C_2O_4)_2(H_2O)_2]^-\cdot HL^-$$

$$cis\text{-}[Cr(C_2O_4)_2(H_2O)_2]^-\cdot HL^- \xrightarrow{k_{an}} cis\text{-}[Cr(C_2O_4)_2(H_2O)(HL)]^{2-} + H_2O$$

$$cis\text{-}[Cr(C_2O_4)_2(H_2O)(HL)]^{2-} \xrightarrow[-H^+]{\text{Fast chelation}} cis\text{-}[Cr(C_2O_4)_2(HL)]^{2-} + H_2O$$

Scheme-II: Mechanism of reaction between carbidopa and [Cr(III) carbidopa complex]

TABLE-2
COMPARISON OF OUTER SPHERE COMPLEX FORMATION CONSTANTS (K_{OS}), ANATION RATE CONSTANTS (k_{an}), $\Delta H^{\#}$ AND $\Delta S^{\#}$ FOR THE ANATION REACTION OF *cis*-DIAQUA-*bis*(OXALATO) CHROMATE(III) WITH OTHER AQUA Cr(III) SYSTEMS

Systems (aqua metal ion/nucleophile)	k_{an} (s^{-1})	K_{OS}	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (J K^{-1} mol^{-1})	Ref.
$Cr(H_2O)_6^{3+}/H_2O$	2.40×10^{-6} (25 °C)		108.6	11.6	[36]
$Cr(H_2O)_6^{3+}$/gly	$(3.34\text{-}6.8) \times 10^{-6}$ (25 °C)		51.9	-42.7	[16]
$Cr(H_2O)_6^{3+}$/ala	0.58×10^{-4} (35 °C)		64.9	-113.6	[20,21,23,39]
$Cr(H_2O)_6^{3+}$/val	2.34×10^{-4} (25 °C)	3.29 ± 0.1	90.4	-21.0	[20,21,23,39]
$Cr(H_2O)_6^{3+}$/L-ornithine	1.70×10^{-6} (40 °C)	18.9	55.8	138 ± 17	[40]
$Cr(H_2O)_6^{3+}$/hydroxyproline	1.00×10^{-4} (40 °C)		72.9	-98.2	[20,21,23,39]
$Cr(H_2O)_6^{3+}$/tryptophan	1.17×10^{-4} (40 °C)		65.6	-112.4	[20,21,23,39]
$Cr(H_2O)_6^{3+}$/methionine	2.22×10^{-4} (35 °C)		61.1	-116.3	[20,21,23,39]
$Cr(H_2O)_6^{3+}$/glutamine	1.81×10^{-4} (40 °C)		52.0	-150.6	[20,21,23,39]
$Cr(H_2O)_6^{3+}$/pyc-3	10.24×10^{-4} (35 °C)	5.42			[41]
$Cr(H_2O)_6^{3+}$/phenylalanine	1.85×10^{-4} (35 °C)		53.6	-141.7	[20,21,23,39]
$[Cr(Salm)(H_2O)_2]^+$/pyc-2	17.60×10^{-4} (25 °C)	8.75	28.3 ± 0.2	201 ± 0.6	[42]
$[Cr(Salm)(H_2O)_2]^+$/pyc-3	18.76×10^{-4} (25 °C)	3.00	38.33 ± 6.5	172 ± 19	[42]
$Cr(H_2O)_6^{3+}$/L-Dopa	1.43×10^{-4} (50 °C)	2.18			[43]
cis-$[Cr(C_2O_4)_2(OH_2)_2]^-$/levodopa	2.75×10^{-4} (50 °C)	0.104	50.13	-68.6	[44]
cis-$[Cr(C_2O_4)_2(OH_2)_2]^-$/carbidopa	1.11×10^{-4} (50 °C)	0.019	65.95	-117.33	This Work

gly = glycine, ala = alanine, val = valine, pyc-2 = pyridine-2-carboxylic acid, pyc-3 = pyridine-3-carboxylic acid, H_2O^* H_2O^{18}, $\Delta H^{\#}$ = activation enthalpy, $\Delta S^{\#}$ = activation entropy

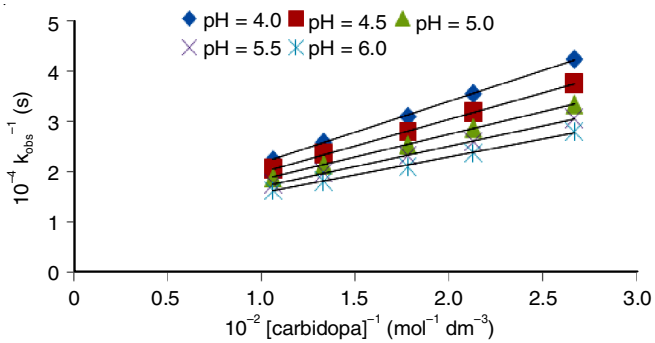


Fig. 4. Plot of 10^{-4} k_{obs}^{-1}/s *versus* 10^{-1} $[HL^-]_T^{-1}$ (mol^{-1} dm^3) at different pH [*cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-]_T$ = 7.5×10^{-4} mol dm^{-3}, I = 0.1 mol/dm^{-3} (KNO_3), Temp. = 50 °C pH varied from 4.0 to 6.0

Characterization of the product complex: A solution of $K[Cr(C_2O_4)_2(H_2O)_2]\cdot 3H_2O$ was mixed with carbidopa solution in 2:1 molar ratio at pH 5.0 and heated at 50 ºC for 6 h. Then, it was dried and the resulting product was recrystallized with ethanol and diethyl ether. FTIR spectrum of carbidopa drug (Fig. 5a) showed a broad peak at 3113 cm^{-1} due to overlapping of aromatic C-H stretching, C-H stretching in CH_2 and CH_3 group. The peaks at 3852 and 3750 cm^{-1} were due to symmetric and asymmetric stretching of water. A sharp peak at 3535 cm^{-1} was due to O-H stretching and N-H stretching, while the bands at 1457, 1529, 1643 cm^{-1} were due to C=C stretching in aromatic ring, C-H bond in CH_2 and CH_3 group, N-H bending, respectively. A band at 1633 cm^{-1} was due to C=O stretching of carboxylate group and 1372 cm^{-1} was due to phenolic O-H bending overlapping. Peaks at 1264 and 1007 cm^{-1} were due to C-O stretching in phenolic group. FTIR spectrum of *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$-carbidopa complex showed a broad peak at 3446 cm^{-1} due to fusion of intermolecular hydrogen bonded O-H, aromatic C-H and N-H stretchings. A small peak at 2283 cm^{-1} was due to overtone band. Shifting of peak due to carboxylate group from 1633 cm^{-1} to 1683 indicates the coordination of carboxylate group to Cr(III). Appearance of sharp peak at 544 cm^{-1} indicates Cr-O stretching frequency and shifting of 3535 cm^{-1} peak to

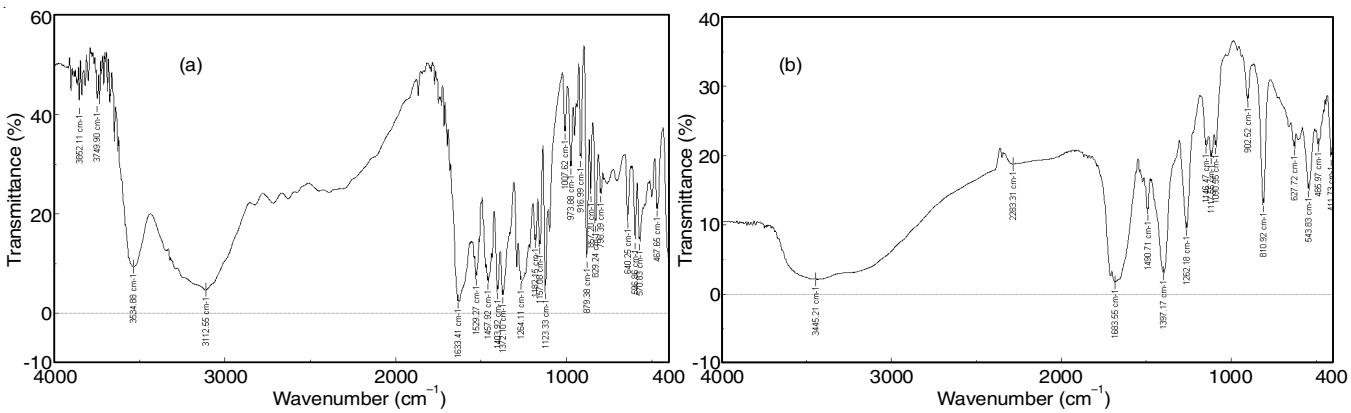


Fig. 5. (a) FTIR spectra of carbidopa (b) FTIR spectra of *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$-carbidopa complex

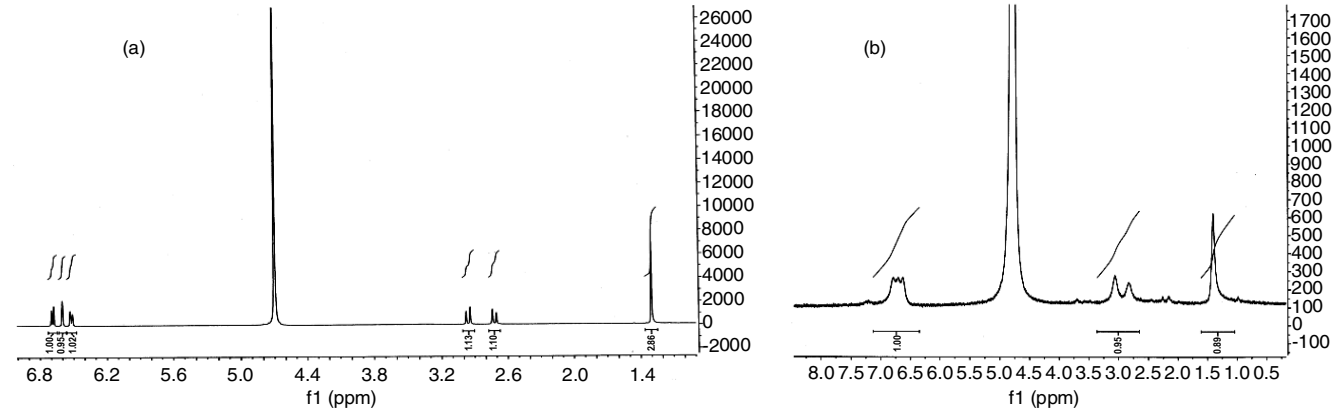


Fig. 6. (a) NMR spectra of carbidopa (b) NMR spectra of *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$-carbidopa complex

TABLE-3
EFFECT OF *cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$-L-Dopa COMPLEX ON ACUTE HALOPERIDOL INDUCED CATALEPSY IN MICE

Group	Treatment	Dose (mg/kg)	Catalepsy				
			15 min	30 min	60 min	90 min	120 min
I	Control	10 mL/kg	1 ± 0.0	1.30 ± 0.08	1.43 ± 0.12	1.39 ± 0.22	1.46 ± 0.31
II	Solvent+HL	10 mL/kg	28 ± 0.98	69 ± 2.79	85 ± 1.21	110 ± 1.09	145 ± 1.12
III	L-Dopa	50	24 ± 1.52 (14.28)	51 ± 1.52[c] (26.09)	55 ± 1.07[c] (35.29)	61 ± 1.22[c] (44.55)	69 ± 1.87[c] (52.41)
IV	L.Dopa+Carbidopa	50 + 25	23 ± 1.41 (17.85)	38 ± 1.38[c] (44.92)	41 ± 1.33 (51.76)	47 ± 1.51 (57.27)	52 ± 1.08 (64.13)
V	L.Dopa+Carbidopa-Cr(III) Complex	50 + 25	18 ± 1.68[a] (35.71)	26 ± 2.11[c] (62.31)	31 ± 1.31 (63.52)	32 ± 1.12 (70.91)	30 ± 1.49[c] (79.31)

Results are expressed in mean ± SEM (n = 6), results are expressed in one-way analysis of variance followed by Dunnet t –test, [a]($p < 0.05$), [b]($p < 0.01$), [c]($p < 0.001$). Values in the parenthesis denote % inhibition of catalepsy.

3445 cm^{-1} is a clear indication of coordination of chromium to nitrogen function (Fig. 5b). All these bands supports the proposed structure of metal complex of chromium(III) with carbidopa (Fig. 2). Assignment of all infrared bands were based on literatures [37,38].

The formation of complex between Cr(III) and carbidopa was further confirmed by ^1H NMR spectra (Fig. 6), which indicates the bonding of carboxylic and amino group to Cr(III) complex. In the product of *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$-carbidopa complex carbidopa moiety was remained undisturbed except at NH_2 group and carboxylic group which was coordinated to chromium. Two doublets corresponding to NH_2 and NH group became two singlet and the peaks were became broad.

Effect of *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$-carbidopa complex on haloperidol induced catalepsy: The study result depicted in Table-3 revealed that combined administration of *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$-carbidopa complex and levodopa significantly ($p < 0.05$) attenuated the cataleptic score compared to untreated and vehicle treated rats. The peak cataleptic score was observed at 30 min after the treatment with 79.31% reduction in cataleptic score after 2 h. In contrast, upo carbidopa and levodopa administration 64.13% reduction in cataleptic score was observed at the end of 2 h. Nevertheless, compared to levodopa and carbidopa combination, *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$-carbidopa complex and levodopa demonstrated enhanced reduction in catalepsy at different time intervals. One plausible explanation of this further reduction in catalepsy observed with *cis*-$[Cr(C_2O_4)_2(OH_2)_2]^-$-carbidopa complex and levodopa combination could be due to increased availability of dopamine in brain as a result of reduced peripheral catabolism of levodopa to dopamine by dopa decarboxylase enzyme.

Conclusion

The present study revealed the kinetics and antiparkinsonian effects of *cis*-$[Cr(C_2O_4)_2(H_2O)_2]^-$ complex with carbidopa, an anti-parkinsonian drug. The FTIR spectrum revealed that Cr(III) ion was bound to carbidopa *via* two sites *i.e.* through carboxylate oxygen and amino group. From the kinetic study, it was established that the complex formation proceeds through a mechanism in which the rate determining step is the step involving the change from an outer sphere to inner sphere complex. In this study, Cr(III) metal complex with carbidopa was screened for its effect in haloperidol induced catalepsy in mice. The cataleptic activity of the product complex showed the effectiveness similar to that of standard drug (levodopa) and further potentiation was observed with the combination of levodopa, carbidopa-Cr(III) complex.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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