Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(7):316-323

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Kinetics and mechanism of oxidation of sulphur containing amino acids by oxo (salen) chromium (V) ion

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ABSTRACT

The oxidation of sensitive sulphide located in a biomolecule such as methionine residue in a peptide or protein, the redox reaction is affected by amino and carboxyl groups present in close proximity to sulphide function. Because of its susceptibility to oxidation, methionine (Met) is thought to play a key role in the migration of unpaired electron in peptides and proteins. By choosing oxo (salen) chromium (V) ion as suitable biomimics for the peptide complexes that are formed during the reduction of Cr (VI) with biological reductants, the oxidation of methionine and ethionine with this in aqueous medium has been investigated by spectrophotometric method. The presence of water facilitates ligation of water to the Cr centre and forms an active oxidant [H_2O - (salen) Cr=O]$^+$ .The reaction is found to be first order each in the oxidant and the substrate. The presence of H^+ ions accelerates the reaction rate. It is found to be first order with respect to H^+ ion. The second order rate constant increases with increase in the water content in the reaction medium. The reaction is carried out at different temperatures and activation parameters are calculated. From the kinetic data and stoichiometric analysis, a mechanism involving direct oxygen transfer from oxidant [H_2O-(salen) Cr=O]$^+$ to the substrate has been proposed as a suitable mechanism for the reaction.

Keywords: Kinetics, oxidation, oxo (salen) chromium (V) ion, methionine, ethionine, methionine sulphoxide.

INDRODUCTION

The metal-salen [*N, N* - bis (salicylidene)-ethylenediamine] complexes are simpler analogues of metal-porphyrin complexes. Salen, a tetra dentate ligand, consists of two nitrogen atoms and two oxygen atoms available for coordination with a metal rather than the four nitrogen donors involved in porphyrin. However, these salen ligands have been shown to form metal complexes that parallel the catalytic activity of metal-porphyrins[1]. For example, iron (III)-salen complexes are similar to iron (III)-porphyrin in that both tetra dentate ligands take up a square planar geometry, often with a fifth ligand (e.g., H_2O) in an apical position and an open sixth coordination site [2]. Most importantly, salens are relatively easy to synthesize from readily available precursors through the condensation of derivatives of salicylaldehyde and ethylenediamine [3]. Further, it is easy to tune the redox properties of oxo (salen) metal complexes by introducing electron donating and withdrawing groups in the para position of phenolic moiety[3,4]. Because of these advantages, oxo (salen) metal complexes were used for the oxygenation of organic substrates, particularly for the sulfoxidation and epoxidation reactions [4]. In recent years, Cr(III)-salen complexes have been widely used as catalysts for the oxidation of organic substrates. Gilheany and co-workers{5] have used these complexes extensively for the epoxidation reactions with PhIO, particularly in the presence of ligand oxides; Adam et al. used them for the chemo selective C-H oxidation, and S. Rajagopal and et al[6,7] for the selective oxidation of sulphides to sulphoxides. Amines and their derivatives are more widely distributed in nature than any other functional group family. The enzymatic oxidation of aromatic amines plays a significant role in many biotransformation processes, such as chemical carcinogenesis [8] and drug metabolism [9]. The oxidation of aromatic amines may produce a large number of products. The most important of them are phenyl hydroxylamine, nitrosobenzene, nitrobenzene, azobenzene, azoxybenzene and oligomers and polymers of aniline [10]. In the past

two decades, relatively long-lived chromium (V) intermediates have been detected in the reaction of Cr (VI) both in vivo and in vitro. Since Cr (V) intermediates are generally considered labile and reactive, this chromium species is considered to be the key species in the mechanism of Cr (VI) carcinogenesis. Of the Cr (V) complexes studied as biomimetics, some noteworthy ones are chromium complexes containing the ligands 2-ethyl-2-hydroxybutyric acid (ehba), salen, picolinic acid (PA), ethylenediamine (en), phenanthroline (phen), and glutathione (GSH). Of these ligand systems, salen type ligands, as a class, consist of flexible and kinetically nonlabile templates wherein both steric and electronic properties of the metal center can be tuned in a synthetically straightforward manner. Further, the Cr (V)-salen complex chosen for the present study mimic Cr-peptide complexes that may form upon intracellular reduction of Cr (VI) by virtue of the mixed nitrogen and oxygen ligand chelation.

Amino acids are susceptible to oxidation by various forms of reactive oxygen species and the oxidation reaction may proceed through one or two electron transfer depending on the nature of the oxidant [11, 12, 13]. However, studies show that when an oxidation sensitive sulphide is located in a biomolecule such as methionine residue in a peptide or protein, the redox reaction is affected by amino and carboxyl groups present in close proximity to sulphide function [14, 15, 16]. Because of its susceptility to oxidation, methionine (Met) is thought to play a key role in the migration of unpaired electron in peptides and proteins. It is suggested that Met can serve as an endogenous antioxidant in proteins and Met oxidation to Met sulphoxide (MetO) has a regulatory function, based on its potential reversion by the enzyme methionine sulphoxide reductase (Msr) [17].

The study on the oxidation of methionine by Cr (VI) has been extensively studied, but no attempt has been made to study the reactivity of Cr (V) complexes [18, 19]. In the present study, the oxidation of methionine and ethionine with oxo (salen) chromium (V) complex has been studied spectrophotometrically in aqueous medium and a suitable mechanism is proposed. The kinetics has been studied at different temperatures and activation parameters are calculated.

EXPERIMENTAL SECTION

MATERIALS

Methionine and ethionine (purity > 99%) were purchased from Sigma – Aldrich and used without further purification. HPLC grade acetonitrile (E.Merck) was used. 70% Perchloric acid (E.Merck) was the source of H^+ utilized to vary the acid concentration in the reaction media. The ligand salen was prepared by refluxing salicylaldehyde and ethylenediamine in ethanol for 2 hrs [20]. PhIO was obtained from iodosobenzene diacetate by hydrolysis as reported in the literature method [21]. The purity of iodosylbenzene was determined by iodometric titration [22]. The chromium (III) – salen complex was prepared by the reaction of hexaaqua chromium (II) with ligand in methanol under nitrogen atmosphere as stated in the previous reports [23].

SYNTHESIS OF OXO (SALEN) CHROMIUM (V) COMPLEX:

A slight excess of iodosylbenzene 0.286g (1.3 mmol) was added to 0.5 g (1.2 mmol) of the chromium (III) complex dissolved in about 50 ml of acetonitrile, whereupon the reaction mixture turned from orange to dark green-brown. The slurry was stirred for an additional 30 min and then filtered to remove the unreacted iodosylbenzene. Anhydrous diethyl ether (200 ml) was slowly added to the dark filtrate in order to precipitate micro crystals, which upon filtration and repeated washing with anhydrous diethyl ether yields oxo (salen) chromium (V) ions as described in the previous reports [21, 23-27]. The structure of oxo (salen) chromium (V) ion is shown in Fig.1.

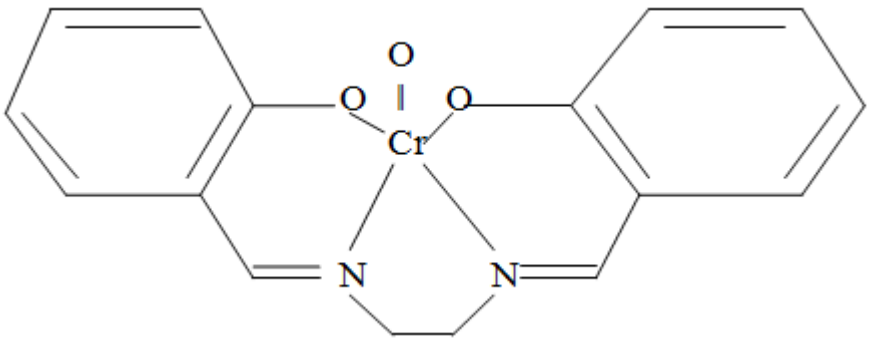


Figure 1 Structure of oxo (salen) chromium (V) ion

SAFETY NOTE:

Caution! Cr (V) compounds are known carcinogens and potential mutagens and hence should be handled with great care and precautions [28].

METHODS AND INSTRUMENTATION:

The kinetic study for the oxidation of methionine and ethionine with oxo (salen) chromium (V) complex were carried out in water. The rate of oxygenation was followed spectrophotometrically under pseudo first order conditions with a substrate : oxidant ratio of at least 10:1 at 303K by measuring the change in the absorbance of the Cr^{V} – H_2O adduct at 610nm in a 1cm cell placed in the cell compartment of LI – 2800 UV – Visible double beam spectrophotometer. Duplicate kinetic runs showed that the rate constants were reproducible to within ± 5%. The kinetic studies at different temperatures were carried out in Agilent diode array spectrophotometer.

RESULTS AND DISCUSSION

Kinetics were followed under pseudo-first order conditions at 30 $\pm$ 0.1 ºC spectrophotometrically by measuring the absorbance of Cr^{V} – H_2O adduct at 610 nm in a 1cm cell placed in the cell compartment of LI – 2800 UV – Visible spectrophotometer. Rate constants at different initial concentrations of methionine and ethionine are presented in Table 1and Table 2. Plot of logarithm of optical density against time yielded straight line indicating first order dependence in substrates and from the gradients of plots, pseudo first – order rate constants, k_1 were calculated by the method of least squares.

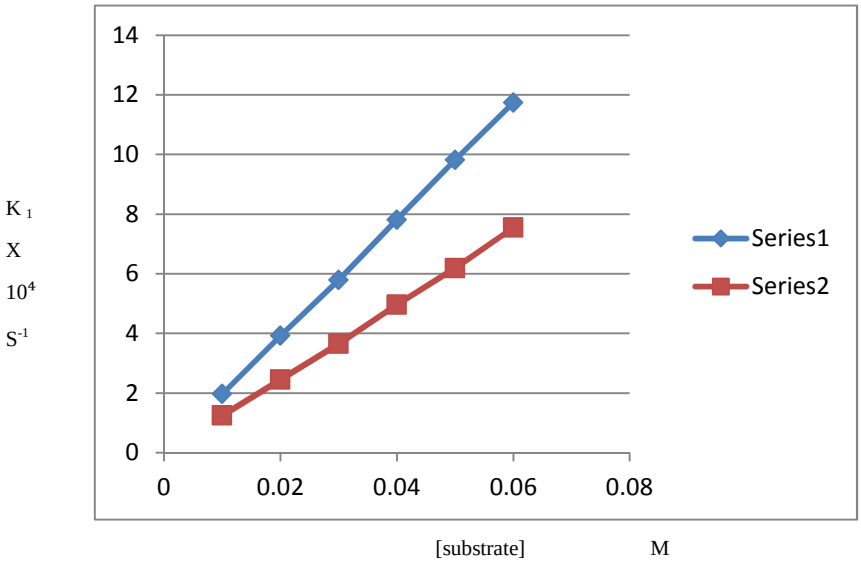
Table 1 Pseudo first order rate constant k_1 and second order rate constant k_2 for the oxidation of methionine by oxo(salen)chromium(V) complex in aqueous medium at 303K
[Oxo (salen) Cr (V)] = 0.001M and [$HClO_4$] = 0.01 M

[Met] M	K_1 X 10^4 S^{-1}	K_2 X 10^2 $M^{-1}S^{-1}$
0.01	1.97±0.09	1.97±0.09
0.02	3.92±0.08	1.96±0.08
0.03	5.79±0.07	1.93±0.07
0.04	7.81±0.08	1.95±0.08
0.05	9.82±0.09	1.96±0.09
0.06	11.74±0.07	1.96±0.07

Table 2 Pseudo first order rate constant k_1 and second order rate constant k_2 for the oxidation of ethionine by oxo(salen)chromium(V) complex in aqueous medium at 303K
[Oxo (salen) Cr (V)] = 0.001M and [$HClO_4$] = 0.01 M

[Eth] M	K_1 X 10^4 S^{-1}	K_2 X 10^2 $M^{-1}S^{-1}$
0.01	1.25±0.06	1.25±0.06
0.02	2.45±0.05	1.23±0.05
0.03	3.65±0.04	1.22±0.04
0.04	4.96±0.05	1.24±0.05
0.05	6.19±0.04	1.24±0.04
0.06	7.55±0.06	1.26±0.06

Figure 2
K_1 Vs [Substrate]



The linear relationship between k_1 and [substrate] as shown in Figure 2 and constant k_2 ($k_2 = k_1$ / [substrate]) as shown in Table 1 point out the first order dependence in the substrate. In Figure 2, series 1 is for methionine and series 2 is for ethionine. The order with respect to oxo(salen)chromium(V) ion in the concentration range $1.0X10^{-3}$ – $1.5X10^{-3}$ mol dm^{-3} was found to be unity at constant Met and acid concentrations ([Met] = 0.02 mol dm^{-3}, acid [$HClO_4$] = 0.1 mol dm^{-3}).

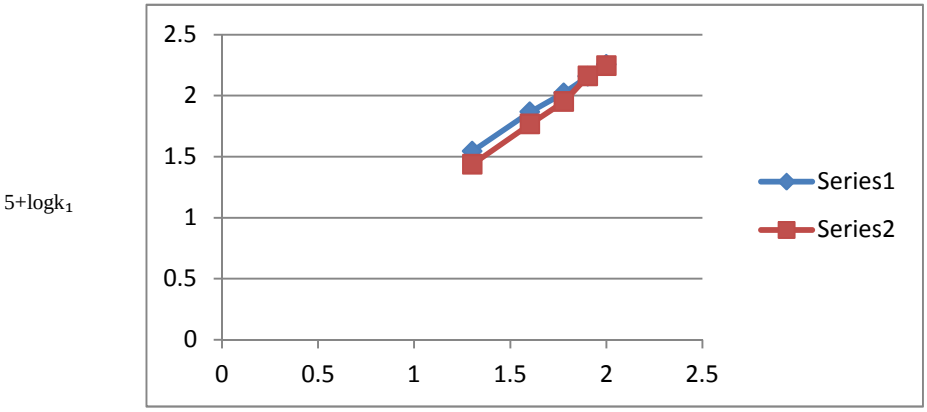
The effect of changing perchloric acid concentration was studied between 0.02 to 0.1 mol dm^{-3} at constant oxidant (0.001M) and substrate (0.01M) concentrations. The second order rate constants for different [H^+] are given in Table 3. The order with respect to acid was found to be 1 from the log – log plot of the rate constants and [H^*] as shown in figure 3. In this figure series 1 is for methionine and series 2 is for ethionine.

Table 3 Effect of changing the [H^+] on the second order rate constants for the oxidation of methionine and ethionine by oxo (salen) chromium (V) complex in aqueous medium at 303K
[Oxo (salen) Cr (V)] = 0.001M [Met] = 0.01 M and [Eth] = 0.01 M

[H^+] M	K_2 X 10^2 M^{-1} S^{-1}	
	Methionine	Ethionine
0.02	3.49±0.05	2.72±0.08
0.04	7.34±0.09	5.82±0.06
0.06	10.49±0.06	8.9±0.05
0.08	14.32±0.08	14.4±0.07
0.1	17.92±0.07	17.4±0.09

Figure 3

Log K_1 Vs
Log [H^+]



The effect of changing the solvent composition on the rate of the reaction has been carried out. The data indicates, with the increase in water content the second order rate constant also increases (Table 4).

Table 4 Effect of changing the solvent composition on the second order rate constants for the oxidation of methionine and ethionine by oxo (salen) chromium (V) complex in aqueous medium at 303K
[Oxo (salen) Cr (V)] = 0.001M, [Met] = 0.01 M, [Eth] = 0.01 M and [$HClO_4$] = 0.01 M

% CH_3CN	% H_2O	K_2 X 10^2 M^{-1} S^{-1}	
		Methionine	Ethionine
30	70	0.14±0.05	0.08±0.08
40	60	0.64±0.09	0.28±0.06
50	50	0.77±0.06	0.44±0.05
60	40	1.01±0.08	0.61±0.07
70	30	1.32±0.07	0.76±0.09
0	100	1.97±0.09	1.25±0.06

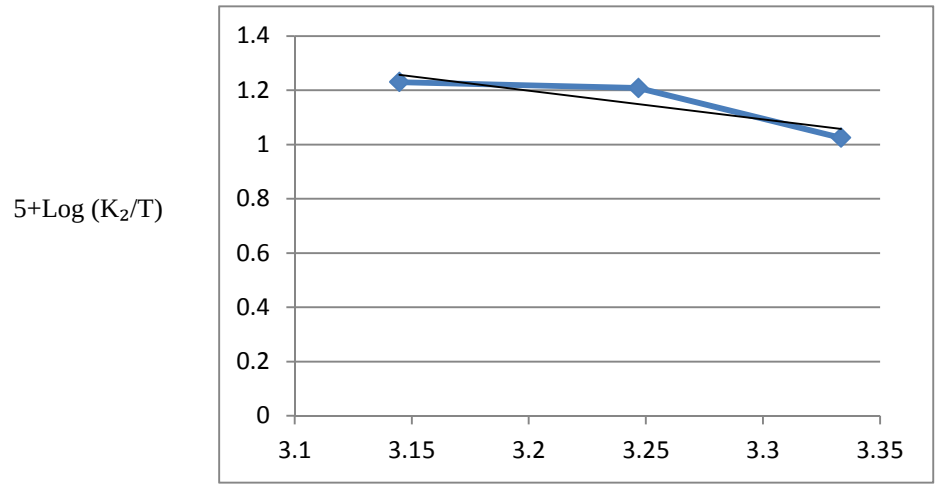
EFFECT OF TEMPERATURE:

The kinetics was studied at three different temperatures viz. 300K, 308K and 318K. The second order rate constants were calculated. The Arrhenius plot of log k_2 versus 1/T was found to be linear. The enthalpy of activation, entropy of activation and free energy of activation were calculated from the plot of log k_2/T versus 1/T using the Eyring relationship. All data are summarized in Table 5. The Eyring plot for methionine is shown in Figure 4.

Table 5 Second order rate constants for the oxidation of methionine and ethionine by oxo (salen) chromium (V) complex at 300K, 308K and 318K and activation parameters
[oxo (salen) Cr (V)] = 0.001M, [Met] = 0.01 M, [Eth] = 0.01 M and [$HClO_4$] = 0.01 M

	K_2 X 10^2 M^{-1} S^{-1}			ΔH*	(-ΔS*)	ΔG*
	T=300K	T=308K	T=318K	KJ Mol^{-1}	JK^{-1} Mol^{-1}	KJ Mol^{-1}
Methionine	3.18±0.09	4.97±0.06	5.4±0.07	20.34	205.27	85.62
Ethionine	2.78±0.06	4.21±0.05	4.7±0.08	20.22	206.88	86

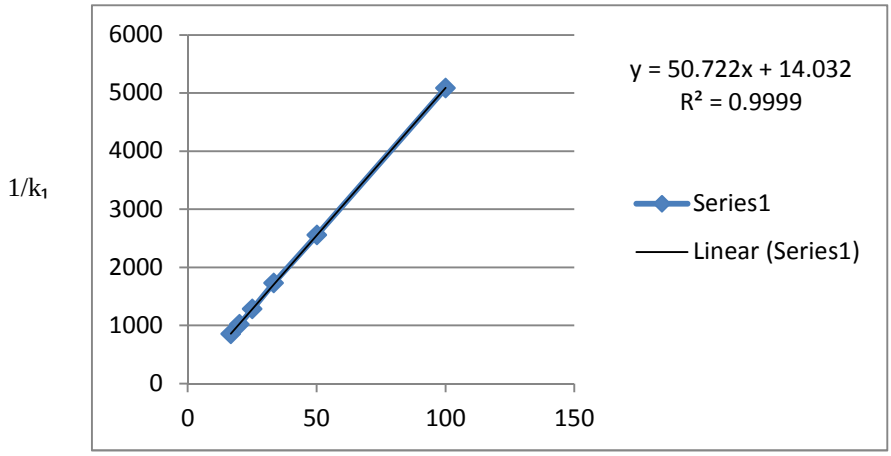
Figure 4
Eyring plot for methionine



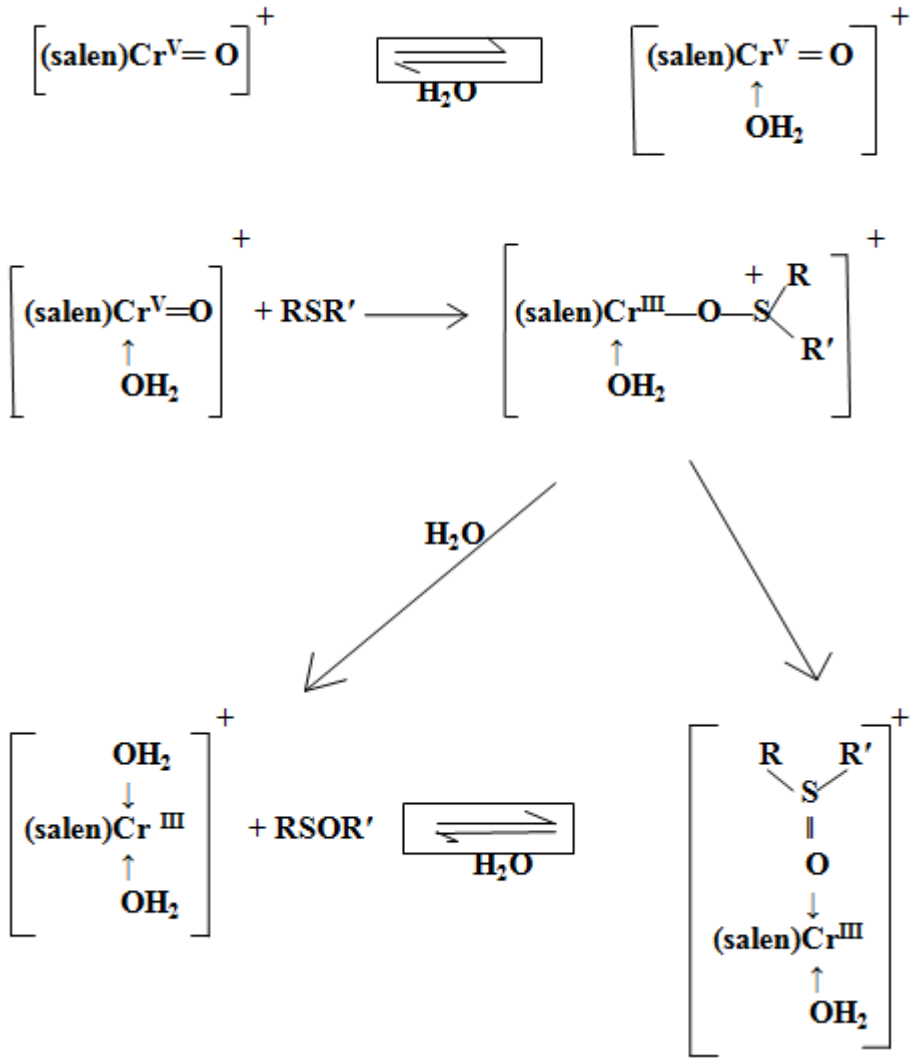
MECHANISM:

From the kinetic data presented above it is inferred that the oxidation of methionine and ethionine with oxo (salen) chromium (V) complex in aqueous medium reacts through a simple kinetics and catalyzed by acid. Upon the addition of water to the reaction a substantial shift in λ_{max} value along with an enormous increase in the ε_{max} value was observed. Such an observation was attributed to the ligation of water to Cr centre. Experiments carried out by Kochi et al. with isotopically labeled oxo(salen)chromium(V) ion undergoes a complete exchange of its oxygen – 18 with ordinary water in acetonitrile solution which further confirm the ligation of water to the Cr centre[24,25]. So the real oxidant in this system will be $[O{=}Cr^{V}$ (salen)-$H_2O]^{+}$ adduct. The kinetics of the reaction was first order in oxidant and in methionine. Recently Venkataramanan et al. has observed a similar trend during the oxidation of sulphoxides and on the ligand oxide assisted oxidation of organic sulphides by oxo (salen) chromium (V) complexes [21, 26]. Hence a mechanism involving electrophilic attack of $[O{=}Cr^{V}$ (salen)-$H_2O]^{+}$ adduct on the sulphur centre of the methionine can be proposed as shown in Scheme 1. A similar mechanism has been found to operate in the oxidation of organic sulphides by oxo (salen) chromium (V) complexes in the presence of various N-oxide donor ligands. However this intermediate molecule may undergo dissociation in two ways. The first one involving the ligation of the oxidized MetO, and the other with an addition of H_2O into the sixth coordination site. Such an insertion of organic molecules into the vacant coordination site has been recently reported by Vairamani et al [29] in which Cr (III)-salen exits with filled fifth and sixth coordination site. Moreover the binding constant for the sulphoxides are found to be very close to the values of water [24-26]. Thus from the previous studies and from the product obtained a mechanism involving oxygen atom transfer by an electrophilic attack of the oxygen of the oxidant at the electron-rich sulphur centre of the methionine can be proposed as the most suitable mechanism. The reaction did not show the polymerization which indicated the absence of free radical intermediate. The stoichiometry of the oxidation of methionine by oxo (salen) chromium (V) was found to be 1:1. Both these support the above mechanism. The results indicate the formation of complex between RSR′ and chromium (V) in presence of perchloric acid. The formation of this complex was proved kinetically by Michaelis – Menten plot i.e., a non – zero intercept of the plot of $1/k_1$ versus 1/ [Methionine] (Figure 5). The mechanism is also supported by the moderate values of ΔH and ΔS. The negative ΔS value indicates that the complex is more stable than the reactants [30]. Recently S.Rajagopal and et al. proposed a similar mechanism for the oxidation of methionine in aqueous acetonitrile medium on the basis of spectral, kinetic and product analysis study [31].

Figure 5 $1/K_1$ Vs 1/ [Methionine]



Scheme 1



CONCLUSION

The kinetics of oxidation of methionine and ethionine by oxo (salen) chromium (V) complex were investigated in aqueous medium by spectrophotometric method at 303 K. The presence of water in the reaction system facilitates the ligation of H_2O to the Cr centre. The reaction was first order with respect to the substrate and oxo (salen) chromium (V) complex. The reaction was catalyzed by perchloric acid. The reaction did not show the

polymerization which indicated the absence of free radical intermediate. The order of reactivity was Met > Eth. The stoichiometry of the reaction was 1:1. A mechanism involving oxygen atom transfer by an electrophilic attack of the oxygen of the oxidant at the electron-rich sulphur centre of the methionine can be proposed as the most suitable mechanism.

Acknowledgements
The author acknowledges University Grants Commission for providing financial support as Minor Research Project [No.F MRP-5335/14(SERO/UGC) dated March 2014]. The author also acknowledges Dr.T.K.Ganesan, Asso.Prof. & H.O.D, PG and Research Department of Chemistry, The American College, Madurai for allowing her to do some of her work in their laboratory.

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