

**RUTHENIUM CATALYZED OXIDATION OF ALANINE AND PHENYLALANINE IN ALKALINE MEDIUM: A COMPARATIVE AND KINETIC STUDY****Hemlata Sahu\* and Sandhya Rani Panda**School of Sciences, MATS University, Raipur, (C.G.), India  
hemlatastryansh16@gmail.com**ABSTRACT**

*The comparative kinetics of Ruthenium(III) catalysed oxidation of L-Alanine and L-Phenyl Alanine by potassium permanganate in alkaline medium has been studied spectrophotometrically using a rapid kinetic accessory. The reactions were found to be of first order with respect to both [oxidant] and [catalyst] and apparently less than unit order with respect to [substrate] & [alkali] respectively. Results suggests the formation of complex between substrate ( alanine and phenyl alanine) and hydroxylated species of ruthenium which further reacts with alkali in rate determining step, resulting in the formation of free radical which again reacts with the alkaline permanganate in a subsequent fast step to yield products in both the cases. Rate constants for different experimental conditions were calculated and there was a good agreement between observed and calculated rate constants. Rate of the reaction is little faster for Alanine than Phenyl Alanine.*

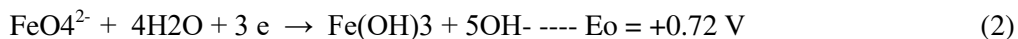
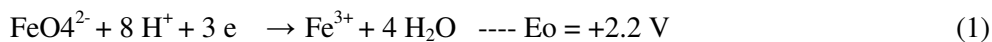
*Keywords: kinetics, oxidation; Rate constant; Ruthenium; Alanine; Phenylalanine; permanganate; spectrophotometrically*

**Introduction:-** Amino acids are building blocks of protein and they also play significant role in metabolism. It can also supply precursors for many endogenous substances such as Hemoglobin in blood. Reactions of amino acids depend upon polar and non-polar substituents contained by amino acids. [1] Uncatalyzed oxidation of alpha amino acids by various oxidants are of particular concern in the field of medicine and biotechnology. Eutrophication taking place in water bodies is caused by various amino acids present in waste water. They can be degraded oxidatively by various oxidants. Alanine is one of the important essential, aliphatic amino acid which is needed for tissue repairing muscle metabolism and coordination and for the maintenance of proper nitrogen balance in our body. It plays key role in glucose-alanine metabolism which enables pyruvate and glutamate to be removed from muscle and find their way to the liver. Any type of alteration in the alanine cycle that increases the levels of serum alanine aminotransferase (ALT) is linked to the risk of type-II diabetes. [2]. Alanine is a source of energy for muscle tissue, brain and central nervous system. Alanine is present in prostate fluid and play a supporting role in prostate health. It produces antibodies and strengthen immune system of our body and helps in metabolism of sugar and organic acids. [3-5]

The oxidation of amino acids is of interest because the products differ depending upon use of oxidant and Catalyst. [6]

Potassium permanganate is one of the most potent and widely used oxidant in synthetic as well as in organic chemistry and also used as disinfectant. Among the six oxidation states of manganese (+2 to +7), +7 is the most potent oxidation state. Potassium permanganate used in redox reactions provides important information of the reactions because the intermediates have sufficiently long life time and easy to identify hence easy to draw possible reaction mechanism. In organic synthesis potassium permanganate is applied extensively especially since the advent of phase transfer catalysis. [7-9]. Oxidation of some  $\alpha$ -amino acids by various oxidants  $\text{KBrO}_3$ ,  $\text{NaIO}_4$ , hexacyanoferrate(III), chloramine-T, NBA, NBS catalyzed by some selective Transition metal ions (Ru, Mn, Os, Ir etc) have been investigated so far. Use of sodium ferrate and potassium ferrate is less in comparison to other oxidant which prompted us to study osmium catalyzed oxidation of Alanine by  $\text{K}_2\text{FeO}_4$ . Potassium Ferrate is best known eco-friendly black-purple compound, which remains stable in moisture free air for long period of time.  $\text{Fe(VI)}$  is a powerful oxidizing agent in aqueous media.  $\text{FeO}_4^{2-}$  is a powerful oxidizing and isostructural with other oxidizing agents like permanganate and chromate.  $\text{Fe(VI)}$  reduces rapidly as well as

exothermally in alkaline medium.[10]. Fe(VI) is a powerful oxidizing agent in acidic and alkaline medium which can be seen from the reduction potentials of following reactions.



Fe(VI) is a strong selective oxidant which oxidizes selectively primary and secondary alcohols to aldehydes (not acids) and ketones respectively [11]

Since oxidation is very slow that's why Suitable catalyst is required, revealed by literature survey.[12-14].

Transition metals are involved to catalyse oxidation of many redox reactions because they show multiple oxidation states. Higher oxidation states of transition metals can be stabilized by chelating with suitable complex agent. There are numerous researches on oxidation of organic compounds by Os(VIII), Ru(IV) , Ir(III), Pd(II) in acidic, alkaline and neutral medium. Among these metals Ru(IV) is known to be an efficient catalyst in several redox reactions particularly in alkaline medium.[15-17]. The formation of different intermediates complexes, free radicals and different oxidation states of Ru makes the study of mechanism of catalysis quite complicated. The kinetics of fast reactions between ruthenate (VII)  $\text{RuO}_4^-$  and manganate  $\text{MnO}_4^{2-}$  have been studied and the reaction is presumed to proceed via an outer sphere mechanism.[18]

Herein we describe the results of the title reaction in order to determine the effect of variation of concentration of oxidant, catalyst and alkali on rate of reaction to arrive at a plausible mechanism.

#### **Experimental:-**

**Materials:** - The ruthenium catalysed reaction of amino acid with permanganate ion in alkaline medium is too fast hence kinetic measurements were performed on a spectrophotometer connected to a rapid kinetic accessory. Stock solutions of amino acids ( Alanine and Phenyl alanine ) were prepared by dissolving the appropriate amount of sample in double distilled water. The potassium permanganate (BDH) solution was prepared and standardized by oxalic acid as described by Carrington and symons [19]. It was standardized by measuring the absorbance on a spectrophotometer with a 1 cm quartz cell at 608 nm. Ruthenium (III) solution was prepared by dissolving  $\text{RuCl}_3$  (s.d. fine chemicals) in  $\text{HClO}_4$ , 0.20 mol  $\text{dm}^{-3}$  mercury was added to the solution to reduce Ruthenium(IV) to Ruthenium(III) solution and stock solution was kept for a day. The concentration of Ruthenium(III) was assayed by EDTA titration [20-21]. All other reagents were of analytical grade and their solutions were prepared by dissolving the requisite amount of sample in double distilled water. Solution of NaOH was used to make medium alkaline and  $\text{NaClO}_4$  to maintain ionic strength of reaction mixture.

**Kinetic measurements:-** All kinetic studies were performed on a spectrophotometer mentioned above which is connected to a rapid kinetic accessory under pseudo first order conditions at constant ionic strength( 0.5 mol  $\text{dm}^{-3}$  ). The kinetics of oxidation of substrate by permanganate ion catalysed by ruthenium(III) was investigated at several initial concentrations of the reactants in alkaline medium. The concentration of oxidant varied (table-1) manifold, keeping concentration of other reactants constant. The reaction was monitored by decrease in absorbance of  $\text{MnO}_4^-$  at 526 nm. The rate of the reaction values (-dc/dt) were calculated from the initial slopes of individual graphs between absorbance versus time. The first order constants ( $k_{\text{obs}}$ ) were evaluated from the plots of  $\log(A_t - A_\infty)$  v<sub>s</sub> time, where  $A_t$ ,  $A_\infty$  are absorbances of permanganate at time t &  $\infty$  respectively. The first order plots in almost all cases were linear to 80%. Completion of the reaction and  $k_{\text{obs}}$  values were reproducible within  $\pm 5\%$ . Similarly concentration of substrate was varied (table-1) by keeping others constant. Effect of concentration of alkali and catalyst were studied by varying their concentration and keeping others constant. Before starting every reaction, reaction mixture of oxidant, catalyst and alkali were thermostated at 35 °C. After completion of reaction, the reaction mixture was concentrated and extracted with ether. The reaction products were isolated using TLC separation techniques.

**RESULTS AND DISCUSSION:-****Table 1: Oxidation of Alaine and Phenyl Alanine by Potassium Permanganate**

Sl.No.	[Ala] & [Phenyl Alanine] *10 <sup>2</sup> mol dm <sup>-3</sup>	[MnO <sub>4</sub> <sup>-</sup> ]*10 <sup>4</sup> mol dm <sup>-3</sup>	[OH <sup>-</sup> ] mol dm <sup>-3</sup>	Ru(III)*10 <sup>6</sup> mol dm <sup>-3</sup>	k <sub>obs</sub> * 10 <sup>2</sup> sec <sup>-1</sup> for Alanine	k <sub>obs</sub> * 10 <sup>2</sup> sec <sup>-1</sup> for Phenyl alanine
1	1	2	0.05	0.2	0.44	0.36
2	2	2	0.05	0.2	0.76	0.62
3	4	2	0.05	0.2	1.13	0.96
4	8	2	0.05	0.2	1.64	1.55
5	10	2	0.05	0.2	1.91	1.72
6	2	.5	0.05	0.2	0.74	0.65
7	2	1	0.05	0.2	0.73	0.63
8	2	2	0.05	0.2	0.73	0.65
9	2	4	0.05	0.2	0.72	0.64
10	2	5	0.05	0.2	0.71	0.64
11	2	2	0.02	0.2	0.34	0.29
12	2	2	0.04	0.2	0.61	0.54
13	2	2	0.05	0.2	0.70	0.64
14	2	2	0.1	0.2	1.14	1.04
15	2	2	0.2	0.2	1.98	1.49
16	2	2	2	0.1	0.39	0.34
17	2	2	2	0.2	0.72	0.66
18	2	2	2	0.4	1.48	1.34
19	2	2	2	0.8	2.98	2.62
20	2	2	2	1.0	3.87	3.32

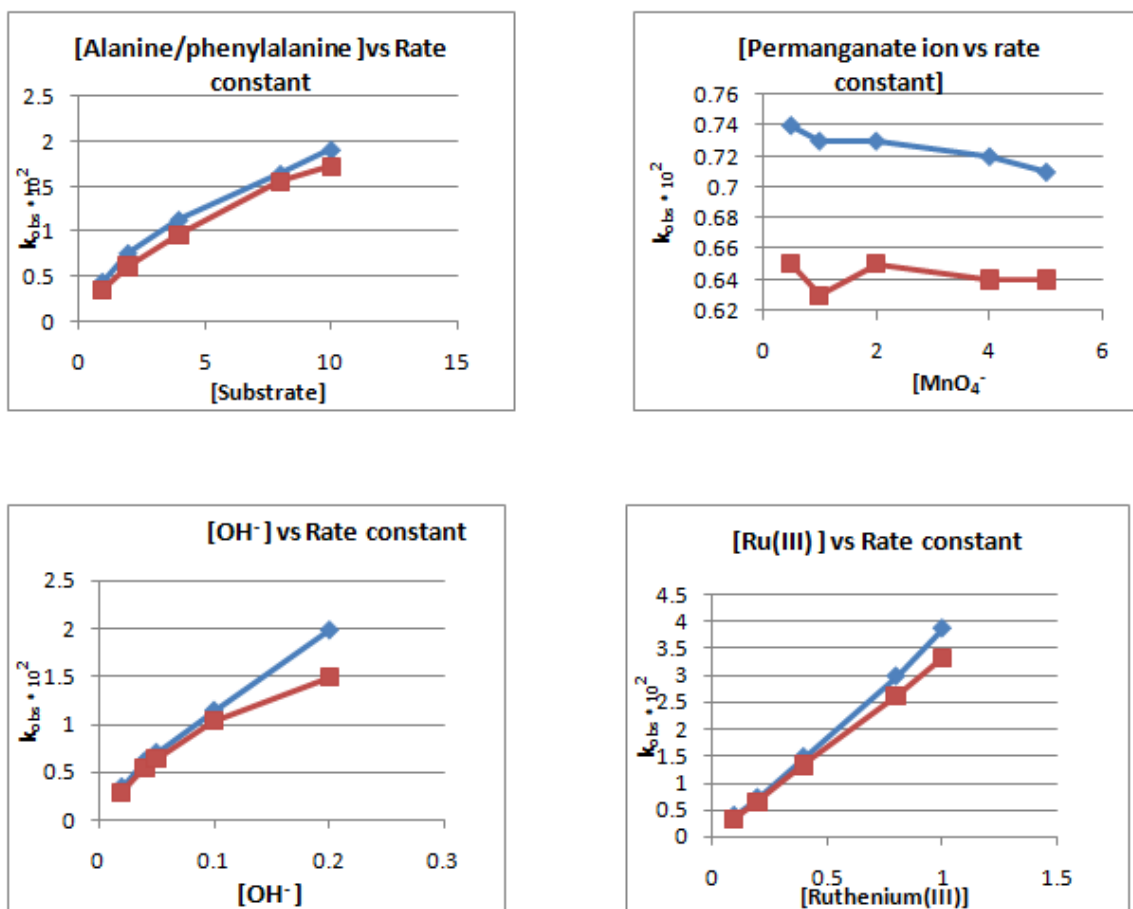
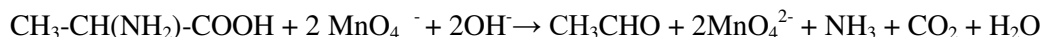


Fig :- Effect of change of concentration on rate constant

#### [A] RESULT ANALYSIS OF ALANINE:-

##### Stoichiometry and Product Analysis:

The reaction products were identified as acetaldehyde [22] by the spot test and ammonia [23] by nessler's reagent and manganate by its visible spectrum. Carbon dioxide was qualitatively detected by passing liberated gas through a tube containing lime water [24]. The product aldehyde was quantitatively estimated to about 78%, which is evidenced by its 2,4-DNP derivative [25]. Nature of the aldehyde was confirmed by its IR spectrum [26], which shows carbonyl stretching at  $1729\text{cm}^{-1}$  and a band at  $2928\text{cm}^{-1}$  due to aldehydic stretching.



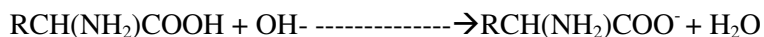
**Reaction Order:-** Order of the reactions were determined by plotting graph between  $\log k$  vs  $\log$  [concentration], by varying concentration of oxidant, catalyst, alkali and catalyst while keeping others constant.

##### Effect of Oxidant, Substrate, And Alkali Catalyst:-

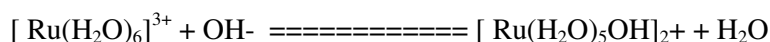
The plots of  $\log(A_\infty - A_t)$  versus time for different initial concentration of  $\text{MnO}_4^-$  are found to be linear and fairly constant  $k$  values indicating first order dependence on  $\text{MnO}_4^-$ . By varying the initial concentration of Alanine and keeping all other concentration and catalyst concentration constant. The rate constant  $k$  increases with increasing concentration of alkali and the order with respect to Ru(III) was also found to be unit.

**Effect of temperature** :-The reaction was measured at four different temperatures with separately varying concentration of substrate. The energy of activation was evaluated from the plot of  $\log k$  versus  $1/T$  from which activation parameters were calculated.

**Discussion** :-Permanganate ion exhibits several oxidation states , pH of the media plays an important role in the reaction. In anionic media Alanine exists as anionic form according to following equilibria.



The reaction between Alanine and permanganate in alkaline medium has a 2:1 stoichiometry with a first order dependence on both  $[\text{MnO}_4^-]$  and ruthenium (III) and less than unit order dependence on both alkali and alanine concentration. In our present study ruthenium forms hydroxylated intermediate species  $[\text{Ru(H}_2\text{O)}_5\text{OH}]^{2+}$ . Rate of the reaction increases with increase in concentration of  $[\text{OH}^-]$ , indicating presence of Hydroxylated species of ruthenium (III) as a reactive species which is shown by the following equilibria.



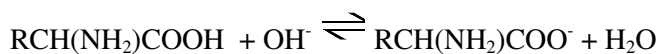
Results of the experiments suggests the formation of a complex between amino acid and the hydroxylated ruthenium(III) species. Such complex formation between substrate and catalyst has also been observed in earlier work [33-37]. The spectral evidence of complex formation was obtained from UV-VIS spectra. Bathochromic shift of 10 nm is observed for alanine. Formation of complex was also proved kinetically by the non zero intercept of plot of  $[\text{Ru(III)}] / k_{\text{obs}}$  versus [alanine].

#### [B] Result analysis of Phenyl alanine :-

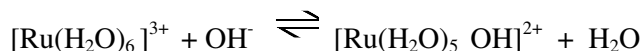
The reaction of Phenyl-alanine with an excess of permanganate and  $.05 \text{ mol dm}^{-3}$  was carried out for about 2 hrs at  $25 \pm 0.1^\circ\text{C}$  under inert atmosphere. After completion of reaction the remaining  $\text{MnO}_4^-$  was analysed spectrophotometrically. Permanganate ion which exhibits several oxidation states is a powerful oxidizing agent in aqueous alkaline medium. pH of the media plays an important role in reaction. Reaction at  $\text{pH} > 12$ , the reaction product of manganese(VII) is stable therefore its further reduction to manganese (VI) might be stopped.

**Stoichiometry analysis**:-Results showed that 2 moles of  $\text{MnO}_4^-$  were consumed by one moles of Phenyl alanine. The stoichiometry of the reaction was found to be 2:1.

**Order of the Reaction**:- It is well known that in aqueous solutions amino acids exist as Zwitterionic forms, however in alkaline medium it exists as anionic form according to the following equilibria.

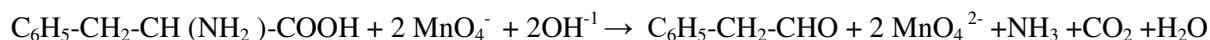


with a first dependence on both the [alkali] and [phenyl alanine]. Under our experimental condition  $[\text{OH}^-] \gg [\text{Ru(III)}]$ . Ruthenium is present as  $[\text{Ru(H}_2\text{O)}_5\text{OH}]^{2+}$ . It was also observed that with increase in concentration of alkali, rate of reaction increases, indicating presence of hydroxylated species of Ruthenium (III) as a reactive species, shown by the following equilibrium which is reported in earlier work.



**Discussion**:-These results suggest the complex formation between substrate and catalyst, which has also been observed in earlier work. The evidence of complex formation between catalyst was obtained in UV-VISIBLE spectra and a bathochromic shift for phenyl-alanine from 233nm to 240nm was observed. The complex formation was also proved kinetically by the non-zero intercept of the plot  $[\text{Ru}^{\text{III}}] / k_{\text{obs}}$  versus  $1/[\text{phenyl alanine}]$ . The observed higher rate constant, modest enthalpy of activation

**Product Analysis:** The reaction products were identified as phenyl aldehyde by boiling point and spot test, ammonia by Nesler's reagent [38-39] and manganate by its visible spectrum. The nature of aldehyde was confirmed by its IR spectrum, which shows carbonyl stretching at  $1729\text{ cm}^{-1}$  and a bond at  $2928\text{ cm}^{-1}$  due to the aldehydic stretching. The products for second series were identified as phenyl acetic acid which was confirmed by boiling point and spots test, ammonia by Nesler's reagent and manganate by its visible spectrum. The nature of carboxylic acid was confirmed by its IR spectrum which showed stretching at  $1657\text{ cm}^{-1}$  and OH stretch at  $2854\text{ cm}^{-1}$ . It was also observed that the aldehyde does not undergo further oxidation under present kinetic condition so it can be concluded that the stoichiometry of the reaction under present kinetic condition is



**Effect of Temperature :-** Activation parameters were calculated by carrying out reaction at four different temperatures. Energy of activation for Phenyl alanine is little higher than Alanine. Log A,  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  are almost in same range.

**Table: 2** Activation parameters for Alanine and Phenyl Alanine:

Activaton Parameters	Alanine	Phenylalanine
Ea kJ mol <sup>-1</sup>	20.1 ± 2.0	20.5 ± 2.0
logA	9.30 ± 1.5	9.31 ± 1.5
$\Delta H^\ddagger$ kJ mol <sup>-1</sup>	17.4 ± 2.0	17.9 ± 2.0
$\Delta S^\ddagger$ J K <sup>-1</sup> mol <sup>-1</sup>	-74.3 ± 10	-74.6 ± 75.2

## CONCLUSION

Amino acids find number of applications in various fields such as biochemical research, microbiology, pharmaceuticals, nutritions etc hence such reactions serve as models for protein oxidation. The reaction between Permanganate ion and amino acids (Alanine & Phenyl Alanine) catalysed by ruthenium(III) chloride occurs with measurable rate in alkaline media. Ruthenium(III) is an efficient catalyst, catalyzing the reaction with a measurable velocity. The reactions are found to be of first order with respect to oxidant and catalyst and less than unit order with respect to substrate in both (Alanine and Phenyl Alanine) the cases. Herein kinetic studies have been used as a tool to know the rate law and reaction mechanism. The derived rate laws are consistent with the observed kinetics and are in excellent agreement with the observed rate of reaction. moderate value of  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  favors electron transfer mechanism. Oxidation products are identified as respective aldehydes in both the cases. Reaction mechanism and reaction products are almost same in both the cases, reaction rate for alanine is little faster for Alanine than Phenyl alanine.

## REFERENCE

1. S.A. Chimatadar, A.K.Kini and S.T. Nandibewoor (2004), *Inorganic Reaction Mechanism* 5, pp 231-244
2. A.B.Nelson, L.David; Cox, M. Michael, principles of biochemistry (4<sup>th</sup> ed). New York;
3. W.H. Free man, 2005; 684-85, 15bno-1767-4339-6
4. Mahant, M.K. and Lalo, D (1990) *J.Chem.Soc.Dalton Trans.* 311
5. Shanmugan, R.M. and Subburamivar, T.V. (1988), *J.Chem.Perkin Trans.* 11, 1341.
6. Bal Reddy, K, Sethuram, B. and Rao, Navaneeth T. (1981) *Indian J.Chem.* 20A, 395
7. Stewart, R (1965) In; Wiberg, K.B, Ed. *Oxidations in Organic Chemistry*, Part A (Academic Press, New York), chapter 1
8. Lee, D.G. (1980) *The Oxidations of Organic Compounds by Permanganate Ion and Hexavalent Chromium* (Open Court, La Salle, IL)

*International Journal of Applied Engineering & Technology*

9. Lee, D.G, Lee, E.J.and Brown, K.C.(1987) *Phase Transfer Catalysis,New Chemistry, Catalysts and applications,ACS symposium series No. 326,( American Chem. Society, Washington DC),p 82*
10. Fatiadi, A.J.(1987) *Synthesis* 106, 85.
11. Sheila et al, *World Journal of Pharmacy and Pharmaceutical Sciences*, 4, Issue-11, 2015
12. D.G. Lee, Chen, T. J. *Org. Chem.*, 1991; 56: 5346.
13. R.J. Audette, J.W. Quail, P.J.; Smith, J *Chem. Soc. Chem. Commun*, 1972; 38.
14. J. Q. Jiang, B. Lloyd/ *Water Reaserch*, 2002; 36: 1397-1408.
15. Sheila Srivastava and Arti Jaiswal, *International Journal of chemistry*, 2012; 1(2): 189-193.
16. Arti Gupta and Anjali Pandey, *Indian J.sci Res.*13: 66-72,2017
17. S.A.Chimatadar, A.K.Kini & Nandibewoor *Indian Journal of Chemistry*, 42A, August2003, pp-1850-1855.
18. S.A.Chimatadar, A.K.Kini & Nandibewoor *Inorganic Reaction Mechanism*, 5, pp-231-244.
19. Carrington A. and Symons, M.C.R.(1956), *j.chem.soc.*(3373)
20. Reddy C.S.and Vijayakumar T.(1995), *Indian J Chem.*(34A,615(CA-72,p 50624,v 1970)
21. Sykes, A.G.*Advances in Inorganic and Radiochemistry* (1967)10, edited by Geneleus H.and Sharpe, A.G.p 321.
22. Feigl F.(1975), *Spot Test in Organic analysis* ( Elsevier, New York) p.195
23. Jeffery, G.H.Bassett, J and Denney R.C.(1996) *Vogel's Textbook of Quantitative Chemical Analysis* 5th Ed( ELBS longman, Essex, UK) p.679
24. Das A.K. and Das M (1994) *J. Chem soc. Dalton Trans.* 589
25. Vogel A.I.(1973) , *A Text book of practical organic chemistry including Qualitative Organic Analysis* 3rd Ed(longmann) p.332
26. Bellamy L.J.(1958) *The IR Spectra of complex organic molecules* 2nd Ed.(Meutheuen and co. London) p.425
27. Langlois,S and Broche, A.(1964) *Bull. Soc.Chin.France* 61, 812
28. Saxena,S.D. and Gupta, K.S.(1977) *J. Inorg.Nucl.Chem.*39,329
29. Srivastava, S.P.and Singh, H.(1971) *J.Indian Chem.Soc.*48, 725
30. Sengupta, K.K. and basu, B.(1977) *Indian J. Chem.*15. 108.
31. Upadhyaya , S.K. and A garwal, M.C. (1980) *Indian J. Chem.* 19,478
32. Chang, R.(1981) *Physical chemistry with applications to Biological Systems* (Mac Millan,New York), p536
33. Langlois,S. and Broche,A.(1966) *Bull,soc.chim.france* 61,812
34. Saxena, S.D. and Gupta, K.S.(1977) *J.Inorg Nud.Chem.* . 39,329
35. Srivastava, S.P. and Singh, H.(1971) *J Indian chem..soc.*48,725
36. Sengupta , K.K. and Basu B.(1977) *Indian J.Chem.*15,108
37. Upadhyay, S.K.and Agarwal, M.C.(1980) *Indian J.Chem.*19,478
38. Feigl.F.(1975) *Spot Test in Organic Analysis* (Elsevier New York),p.212
39. Hugar,G.H. and Nandibewoor , S.T.(1994), *Transition Met.Chem.*19,215