Synthesis of New Transition Metal Peroxo Compounds and Studies on Their Activity in Some Biochemically Relevant Redox Processes

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by

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Dedicated to -

my beloved parents and my son LOY

ABSTRACT

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ABSTRACT

The present thesis deals with the results of studies involving synthesis, assessment of structure and stability, and reactivity of some new peroxo complexes of vanadium and tungsten containing biogenic co-ligands. The thesis also provides an account of the findings of invèstigation on the effect of the compounds on activity of certain enzymes. The contents of the thesis have been distributed over seven chapters. The outline of the thesis is described below.

Chapter 1 presents a brief introduction pertaining to the work incorporated in the thesis. The importance of and the interest in vanadium and tungsten chemistry in general, and peroxo compounds of vanadium(V) and tungsten(VI) in particular are highlighted. Attention has been drawn to the discoveries that remarkably enhanced the importance of vanadium and tungsten and their compounds in biology. Also emphasized in this chapter is the dearth of information concerning the effect of well defined synthetic peroxotungsten compounds on the activity of different enzymes including phosphatases and the relevance of such studies in the context of contemporary interest involving the potential therapeutic applications of peroxo metal compounds as insulin mimetic agents.

This chapter also projects the scope of work on chosen aspects of vanadium and tungsten chemistry.

Chapter 2 describes the details of the methods of the elemental analyses, and instruments/ equipment used for characterization and structural assessment of the newly synthesized compounds. Methods used for studies on the redox activities of the complexes in bromination and interactions with various enzymes are also described herein.

In **Chapter 3** the synthesis and characterization of hitherto unreported mononuclear and dinuclear peroxo complexes of tungsten containing amino acid or dipeptides as co-ligands are described. Moreover, the results of studies on the thermal stability of the compounds, as well as their stability towards decomposition in solutions of a wide range of pH values are reported.

The dinuclear peroxo complexes of tungsten with coordinated cystine of the type $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$, A = Na (**3.1**) or K (**3.2**) have been synthesized from the reaction of A_2WO_4, cysteine and 30% H_2O_2. The pH value of 2.5 attained spontaneously during the reaction was not raised. The oxo-bridged peroxo compounds of W containing dipeptides as heteroligands, $Na_2[W_2O_3(O_2)_4(dipeptide)_2].3H_2O$ [dipeptide = glycyl-glycine (**3.3**) and glycyl-leucine, (**3.4**)] were obtained from the reaction of H_2WO_4 with 30% H_2O_2 and the respective dipeptide ligand. The pH of the reaction medium was adjusted to 2.5 by adding NaOH. The reactions were carried out at an ice-bath temperature ($\leq 4\ ^0C$) and the precipitation of the complexes was brought about by the

addition of ethanol. Similar reactions conducted at pH c.5 afforded the new molecular peroxotungstate(VI) complexes of the type, $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$ [dipeptide = glycyl-glycine (**3.5**) or glycyl-leucine (**3.6**)]. Synthesis of the compounds, in addition to pH, is sensitive to reaction temperature and concentrations of the components.

The compounds were characterized by elemental analysis, spectral and physico-chemical methods including thermal analysis. In the dimeric complexes the two W(VI) centres with edge bound peroxo groups are bridged by an oxo group. The dipeptides occurring as zwitterions are co-ordinated to the metal centers through O(carboxylate) atoms leading to hepta co-ordination around each W(VI). In the compounds **3.1** and **3.2** cystine originating from the oxidation of cysteine forms an additional bridge through its O (carboxylate) atoms between the two metal centres.

Thermal stability of the compounds **3.1-3.6** as well as their stability in solution were determined. Results of molar conductance measurements, electronic spectral studies and estimation of their peroxide content at specific time intervals confirmed that the compounds are highly stable toward decomposition in solutions of acidic as well as physiological pH.

Reported in **Chapter 4** are the synthesis and characterization of a set of new diperoxovanadate(V) complexes with the dipeptides as ancillary ligands of the type, $A[VO(O_2)_2(glycyl\text{-}glycine)(H_2O)].H_2O$, A= Na (**4.1**) or K (**4.2**) and $A[VO(O_2)_2(glycyl\text{-}leucine)(H_2O)].H_2O$, A= Na (**4.3**) or K (**4.4**) and studies on stability of these complexes in solution.

The compounds were isolated from the reaction of V_2O_5 with H_2O_2 and the respective dipeptide at near neutral pH of 5.5. Results of elemental analysis, thermal analysis, magnetic susceptibility, and spectral studies were in complete agreement with the formula assigned to the complexes. The complexes contain side-on bound peroxo groups and a dipeptide zwitterion as co-ligand, binding the metal centre unidentately through O (carboxylate) atom. The compounds exhibit remarkable stability in solution of pH ranging fom 3.5 to 8.0.

Chapter 5 deals with the results of investigations on the effect of the enzyme catalase on the peroxotungsten and peroxovanadium compounds, $A_2[W_2O_3(O_2)_4(\text{cystine})].4H_2O$, A = Na (**3.1**) or K (**3.2**), $Na_2[W_2O_3(O_2)_4(\text{dipeptide})_2].3H_2O$ [dipeptide = glycyl-glycine (**3.3**) and glycyl-leucine, (**3.4**)], $[WO(O_2)_2(\text{dipeptide})(H_2O)].3H_2O$ [dipeptide = glycyl-glycine (**3.5**) or glycyl-leucine (**3.6**)], $A[VO(O_2)_2(\text{glycyl-glycine})(H_2O)].H_2O$, A= Na (**4.1**) or K (**4.2**) and $A[VO(O_2)_2(\text{glycyl-leucine})(H_2O)].H_2O$, A= Na (**4.3**) or K (**4.4**) their oxidizing ability with respect to reduced glutathione(GSH), and their activity as inhibitors of the enzyme alkaline phosphatase. Comparisons between the two sets of peroxovanadium and peroxotungsten compounds in analogous co-ligand environment could be drawn with respect to their tested properties.

The effect of catalase on complexes was studied by estimating their peroxide content in a solution containing catalase and phosphate buffer (pH 7.0) at specified time intervals. On incubation with catalase, each of the compounds **3.1- 4.4** was found to be degraded gradually with the loss of peroxide. From the rates of degradation of the

compounds under the effect of catalase, it was evident that the synthesized peroxovanadium and peroxotungsten complexes are 20-50 fold weaker substrates to catalase as compared to H_2O_2, its natural substrate.

Oxidizing capacity of peroxometallates with respect to reduced GSH, were tested by the method of Ellman. The peroxovanadium compounds (**4.1-4.4**) efficiently oxidized GSH to GSSG, a reaction in which the stoichiometry of 4:1 for GSH oxidized to peroxovanadium compound was recorded, as expected from the presence of two peroxo groups in these compounds. Significantly, GSH oxidizing ability of peroxotungsten compounds **3.1-3.6** irrespective of being monomeric or dimeric, was limited to ca. 50% of that expected on the basis of the total number of peroxo groups present in these complexes.

The effect of the newly synthesized peroxo-metal complexes **3.1-4.4** upon ALP activity of rabbit intestine alkaline phophatase was tested by employing established enzyme assay system and p-nitrophenylphosphate (p-NPP) as substrate. To quantify the inhibitory potential of the molecules, the half-maximal inhibitory concentration (IC_{50}) for each inhibitor which gave rise to a 50% suppression of the original enzyme activity and the enzymatic rate ratios V_0/Vi where V_0 is the uninhibited rate and Vi is the rate of the enzymatic reaction inhibited by the complexes and other species were determined. The results demonstrated that each of the peroxovanadium and peroxotungsten compounds, despite having 7 co-ordinated metal centres induce strong inhibitory effect on alkaline phosphatase activity with potency higher then that of the free ligand, tungstate, vanadate, peroxotungstate or peroxovanadate. The inhibitor potencies of the compounds appear to be sensitive to the nature of co-ligand environment.

Chapter 6 deals with the results of investigations on the reactivity of the mononuclear and dinuclear peroxotungsten complexes, $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$, A = Na (**3.1**) or K (**3.2**), $Na_2[W_2O_3(O_2)_4(dipeptide)_2].3H_2O$ [dipeptide = glycyl-glycine (**3.3**) and glycyl-leucine, (**3.4**)] and $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$ [dipeptide = glycyl-glycine (**3.5**) or glycyl-leucine (**3.6**)] in oxidative bromination.

The compounds efficiently oxidized bromide to a bromination competent intermediate in phosphate buffer at physiological pH. The bromination of phenol red to bromophenol blue was employed to investigate the bromination activity of the complexes **3.1-3.6** in solution. Addition of freshly prepared aqueous solution of the compound to the standard reaction of bromide in phosphate buffer with phenol red as trap for oxidized bromine resulted in gradual color change of the solution from yellow to blue. The spectrum recorded showed a peak at 592 nm characteristic of the product bromophenol blue and a decrease in absorbance of the peak at 433 nm due to loss of phenol red.

The bromination activity of each of the monomeric diperoxo and dimeric tetraperoxo complexes was observed to be restricted to approximately half of that expected on the basis of the total peroxide content of these complexes. Decrease or increase in concentrations of the compound, substrate or KBr in the reaction solution had no effect on this feature. While the initial addition of H_2O_2 to the reaction solution had no observable effect on the initial rate of bromination, a revival of the bromination activity on addition of H_2O_2, to a spent reaction mixture that contained excess bromide and substrate was noted. Exogenous hydrogen peroxide is therefore required in order to obtain a catalytic cycle. Based on the results a mechanistic pathway implicating the

formation of an inactive monoperoxo tungsten intermediate has been formulated. The compounds were also found to be active in bromination of several activated aromatic substrates in aqueous – organic media at ambient temperature.

Diperoxovanadium compounds, **4.1- 4.4** were found to be totally inactive in bromination under identical conditions.

In **Chapter 7**, the notable points emerging out of the present investigation are summarized and conclusions are drawn on the basis of the results of the work undertaken.

The major part of the results of studies described in **Chapters 3 - 6** have been either published or accepted for publication.

Chapters **3** and **5**

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Chapters **4** and **5**

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Chapter **3** and **6**

Polyhedron, 2006, **25,** 3501-3508.

Declaration

I hereby declare that the thesis entitled "***Synthesis of New Transition Metal Peroxo Compounds and Studies on Their Activities in Some Biochemically Relevant Redox Processes***" being submitted to the Department of Chemical Sciences, Tezpur University, is a record of original research work carried out by me. Any text, figures, results or designs that are not of own devising are appropriately referenced in order to give credit to the original author(s). All sources of assistance have been assigned due acknowledgement I also declare that neither this work as a whole nor a part of it has been submitted to any other university or institute for any other degree, diploma or award.

Pankaj Hazarika

Date: 20.09.2007 (Pankaj Hazarika)

Place: Tezpur.

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I certify that the thesis entitled "***Synthesis of New Transition Metal Peroxo Compounds and Studies on Their Activities in Some Biochemically Relevant Redox Processes***" submitted by Mr. Pankaj Hazarika for the degree of Doctor of philosophy of Tezpur University, embodies the record of original investigation carried out by him under my supervision. He has been duly registered, and the thesis presented is worthy of being considered for the Ph.D. Degree. This work has not been submitted for any degree of any other university.

Date: 20.9.07

Place: Tezpur

N. S. Islam
Signature of the Supervisor

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List of Abbreviations

ALP	alkaline phosphatase
ADPV	alkali diperoxovanadate
AH	acetylene hydratase
AOR	aldehyde ferredoxin oxidoreductase
DTNB	dithionitrobenzoic acid
DPV	diperoxovanadate
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
FDH	formate dehydrogenase
FMDH	n-formylmethanofuran dehydrogenase
GSH	glutathione
GSSG	glutathione oxidised
HIV	human immunodeficiency virus
HMPA	hexarnethylphosphoric triamide
HPLC	high Performance Liquid Chromatography
gly-ala	glycyl-alanine
gly-gly	glycyl-glycine
gly-leu	glycyl- leucine
IC_{50}	half-maximal inhibitory concentration
IR	infra red
insRTK	insulin receptor tyrosine kinase
LMCT	ligand to metal charge transfer
MPV	monoperoxovanadate
NAD	nucleotide adenine dinucleotide
NADH	nucleotide adenine dinucleotide reduced form
ndt	2,3- naphthalenedithiolate

NMR	nuclear magnetic resonance
NTA	nitrilotriacetic acid
O-phen	o-phenanthroline
pic	picolinato
p-NPP	p-nitrophenyl phosphate
p-NP	p-nitrophenol
pV	peroxovanadate
pW	peroxotungstate
PTPase	phosphotyrosine phosphatase
PTC	phase transfer catalyst
RNA	ribonucleic acid
TG	thermogravimetry
tacn	triazacyclononane
DTG	differential thermogravimetry
TMB	1,3,5-trimethoxybenzene
V-BPO	vanadium bromoperoxidase
V-HPO	vanadium haloperoxidase

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CHAPTER 1

Introduction

1.1 VANADIUM AND TUNGSTEN – HISTORY AND OCCURRENCE

Vanadium, a group V transition element with outer electronic configuration $3d^3 4s^2$, was first discovered in 1801 by a mineralogist Andres Manuel del Rio in a brown lead mineral from Mexico [1,2]. Owing to the varied colours of its compounds, he first named it as *panchromium,* but subsequently changed the name to *erythronium* (red) because of the red colour of its salts when treated with acids[1]. However, vanadium was rediscovered [3] in the year 1831 by the Swedish chemist Sefström after del Rio had, mistakenly, withdrew his discovery. The beautiful colours of vanadium minerals prompted its naming as *vanadin* by its discoverer after *Vanadis*, the Scandinavian goddess of beauty.

The average abundance of vanadium in nature [4-6] is approx. 0.02%. In ocean it is the second most abundant transition element (50 nM)[7]. The minerals patronite (a complex sulfide), *carnotite* [$K(UO_2)VO_4.3/2H_2O$], *vanadinite* [$Pb_5(VO_4)_3Cl$] and *roscoelite* are important sources of vanadium[2,4]. It is generally the most abundant trace metal in crude oils and in shales[2,8] where it is present in the form of organic complexes.

Vanadium compounds are also found in traces in biosphere[5,9]. It is normally present at very low concentrations in virtually all cells in plants and animals [10]. In mammals it is an ultra trace element, which is widely distributed in tissues [11]. However, the nature of vanadium species and its role in these bio-systems remain unclear [12]. Vanadium is found naturally associated with two types of enzymes, haloperoxidases found in marine

organisms [13] and certain nitrogenases of nitrogen-fixing bacteria (*Azotobacter*) [14]. Some accessory foods such as black pepper, tea leaf, cocoa powder and some mushrooms contain relatively high amounts of vanadium.

Tungsten belongs to Group VI of the periodic table having the ground state electron configuration [Xe] $4f^{14}5d^{4}6s^{2}$ [15-17]. In 1758, the Swedish chemist and mineralogist, Axel Fredrik Cronstedt, discovered and described an unusually heavy mineral that he called "tungsten", which is Swedish for "heavy stone". He was convinced that this mineral contained a new and, as yet undiscovered, element[18,19]. It was not until 1781 that a fellow Swede, Carl Wilhelm Scheele, succeeded in isolating the oxide (tungsten trioxide) [15,18,20]. The name "wolfram" for the metal in favour to tungsten[15,20] was proposed by Berzelius (1816) to describe the oxides of tungsten. In 1847, R. Oxland took out a patent for the manufacture of sodium tungstate and tungstic acid, which forms the starting point of the metallurgy of tungsten. Thus Oxland was the real founder of the tungsten chemistry[18,20].

Unlike vanadium, which is ubiquitous in nature[21], tungsten is relatively scarce in natural environment [22,23]. The average abundance of tungsten on earth is approximately 1ppm (microgram/g)[15]. In ocean water it's abundance is 2.0×10^{-5} mg/litre [24]. W is ranked 54th in natural abundance [25]. Tungsten occurs in the natural state only in the form of chemical compounds with other elements. Although more than twenty tungsten bearing minerals are known, only two of them are important for industrial use, namely wolframite (Fe, Mn)WO_4 and scheelite $CaWO_4$. Presence of tungsten in some biological microorganism has also been established with the recent discovery of several tungstoenzymes [26].

Tungsten and its alloys are used extensively for making filaments for electrical lamps and electron tubes[15]. The element has the highest melting (3380 ^{0}C) point and lowest vapor

pressure of all metals, and at temperatures over 1650 K has the highest tensile strength owing to which W is used in various high temperature applications[15-17]. Vanadium, in addition to its utility as oxidation catalysts in industrial processes [27,28] is now considered essential in trace quantities[29-31] has known therapeutic applications [5,6,10,32-48] and is toxic in excess [48-52].

1.2 THE GROWING IMPORTANCE OF VANADIUM AND TUNGSTEN IN BIOLOGY

Several major discoveries on biological effects of vanadium [8,10-14, 53-56,57] over the last two decades, some of which are listed in Table 1.1, raised the status of this element from that of a low adventitious contaminant to one of high biological relevance. The inhibitory effect of vanadate towards phosphatase was established in 1977 when Cantley and co-workers[58] reported that vanadate is a potent inhibitor of Na, K-ATPase. This was the beginning of understanding of the potential of vanadate in enhancing effectiveness of a variety of phosphate esters, including phosphoproteins, by inhibiting their hydrolysis. It was shown in 1980 that vanadate and vanadyl had the insulin-mimetic action of enhancing glucose oxidation in rat adipocytes [59,60]. These reports marked the resurgence of interest in finding anti-diabetic vanadium compounds with low toxicity [5,6,10,37-40,43-46,61-64] and identification of peroxovanadates as possible active compounds that activate directly the cascade of enzymes that normally follows activation of insulin-receptor [65] (Table 1.1). Ramasarma and co-workers found in 1981 that oxidation of NADH by dioxygen was enhanced several fold in liver plasma membranes on addition of vanadate and this H_2O_2-generating oxygen-consumption reaction, was inhibited by superoxide dismutase [66]. This unexpected and

unusual effect led to the discovery of peroxo-vanadate intermediates that act as selective oxidants, and spurred research on the redox profile of vanadium. A major breakthrough was the demonstration in 1985 that oral administration of vanadate solutions lowered blood sugar in diabetic rat [33]. Finally, with the discoveries of proteins containing bound vanadium as a native constituent and essential for the activity of a bromoperoxidase [13] in a marine alga, in the year 1983, and of nitrogenase in *Azotobacter* [14] the biological role of vanadium has been firmly established.

The pharmacological value of metavanadate was recognized a century ago in France and it was acclaimed as "*Panacee Universelle*" for treatment of a number of diseases as diverse as anemia, tuberculosis, syphilis and diabetes[67],[68]. A metavanadate containing tonic (neogadine) is available in the market in India. Most food materials used for human consumption contain vanadium in concentrations [31] below 0.1 μg/g. Dietary supplement of vanadate increases its tissue content which is stored in a non-toxic form[69]. However, pharma-

Table 1.1 *Resurgence of interest in biological actions of vanadium*[56]

Reaction/Parameter	Vanadium	Effect/Locale	Reference
Na,K-ATPase	vanadate	inhibition	58
Insulin-mimic	vanadate	blood glucose$^-$	60
Insulin-mimic	vanadyl	blood glucose$^-$	59
Noradrenaline-mimic	vanadate	arterial contraction	70
NADH-V reductase	vanadate	plasma membrane	71
NADH-O_2 oxidation	polyvanadate	plasma membrane	66
Bromoperoxidase	vanadate	marine alga	13
Nitrogenase mutant	vanadate	*A. vinelandii*	14

-cological potential of vanadium has been systematically explored only in the last decade or so [33]. There is a great need for an effective oral anti-diabetic agent, since none of the available insulin is orally effective. No other metal salts have rivaled vanadium compounds as effective insulin substitutes [61]. Yet, they have limited clinical usefulness so far due to several factors including toxicity of the metal [63].

The discovery of tungstoenzymes in thermophilic bacteria and hyperthermophilic archea [26] is considered as one of the most exciting developments in contemporary bioinorganic chemistry [26,72,73]. The possibility of the existence of other organisms that might utilize this element is yet to be explored [26]. Tungsten was till recently treated only as a molybdenum antagonist, since replacement of Mo by W leads to inactivation of Mo containing enzymes[74] The atomic and ionic radii and the chemical properties of tungsten are very similar to those of molybdenum. They can catalyze reactions such as hydroxylation of carbon centres under more moderate conditions than are required by other systems[75]. It is therefore surprising that while the essential role of molybdenum in biology has been known for decades and molybdoenzymes are ubiquitous [74,75] yet, the evidence for involvement of W in biological systems could be obtained only recently.

The first major breakthrough in this area came in the year 1983 with the report on isolation and purification of the first naturally occurring tungstoenzyme from one of the acetogens although, it has been known since 1970s that tungstate stimulated the growth of certain acetate and methane-producing microorganisms[76-83]. During the last decade the partial characterization of a number of oxygen-sensitive, pterin-containing tungsten enzymes from thermophiles has stimulated a renewed interest in the bioinorganic chemistry of tungsten[84-86]. At present, more than a dozen tungstoenzymes have been purified[87,88] and the crystal

structure of one of them has been determined.[89] The physiological roles of these enzymes are fundamental, and include the catalysis of key steps in carbon, nitrogen and sulfur metabolism [26,90,91]. Tungstoenzymes are grouped into three categories [87,89,92]. Members of the AOR and F(M) DH families catalyze redox reactions, whereas, AH type catalyze hydration of acetylene. Summarized in Table 1.2 are the types of tungstoenzymes and the reactions catalyzed by them[26,93]

Table 1.2 *Representative examples of the reactions catalyzed by Tungstoenzymes*[26,93]

Enzyme	Reactions catalyzed	Metal and cofactor content, mol/mol protein	Ref.
Formate dehydrogenase	$HCO_2^- \rightleftharpoons CO_2 + H^+ + 2e^-$	W (2) Se (2) Fe/S (20-40)	94
Formaldehyde:ferredoxin -oxidoreductase	CO_2 activation	W (4) FeS (4)	95, 96
Aldehyde: oxidoreductase	$RCHO + H_2O \rightleftharpoons RCO_2H + 2H^+ + 2e^-$	W (2) FeS (4)	97
Acetylene hydratase	$HC \equiv CH + H_2O \rightleftharpoons CH_3CHO$	W (0.5) Fe (3) S (4)	98

Quite exciting is to draw attention to another interesting development, which enhanced the awareness of biochemical importance of tungsten and its compounds is the finding of Goto et al in a 1992 on the ability of tungstates[99], like vanadates to mimic the

biological action of insulin[100]. Moreover, enzyme inihibitory effect of tungstates on protein phophatases activity was documented by then[101]. The known insulin mimetic effects of tungstate[101] led to polyoxotungstate clusters being evaluated as insulin mimetics in animal models [102]. In addition, compounds of tungsten such as polyoxotungstates, particularly silicotungstates have been recently reported to show antiviral activity [103,104] and were found to be potent inhibitors of HIV reverse transcriptase and RNA-dependant DNA polymerase[104]. Despite these important findings and the fact that toxicity of W is relatively low, there appears to be very few studies devoted to the biochemical studies or exploration of therapeutic potential of compounds of tungsten[100,105-108]. Concomitant with renewed biological interest there has been an increasing interest in elucidating the chemistry of tungsten and vanadium complexes as their co-ordination chemistry play a central role in the interaction with biomolecules[7,9,100,109-113] as well as in catalytic oxidations [114-122].

1.3 SELECTED ASPECTS OF TUNGSTEN AND VANADIUM CO-ORDINATION CHEMISTRY

Both vanadium and tungsten have a chemical versatility that is useful to biological systems and are redox active under physiological conditions[26,109]. In order to understand how these metals might function in relatively complex biomolecules as well as their role in catalytic oxidations, it is incumbent on us to understand its basic co-ordination chemistry with simpler ligands.

The co-ordination chemistry of vanadium has achieved a special status in the last decade [123] owing mainly to the model character of many vanadium complexes for the

biological function of vanadium [46,112,115,116,124-130], the use of oxo V complexes in oxidation and oxo transfer catalysis[116,131-133] and potential medicinal applications [6,7,10,43-46,134]. While vanadium can exist in at least six oxidation states, only the three highest, i.e. +3, +4, and +5, are important in biological systems[135]. Vanadium(IV) and vanadium(V) oxidation states are more common and are stable under ordinary conditions[135]. Vanadium studies remained in low profile due to the complexity of its chemistry in solution [9,136]. The potential for redox interplay, whether V(V)/V(IV) or V(IV)/V(III), increases the versatility of this element in the biological milieu [137].

The majority of V(IV) compounds contain the VO^{2+} unit (vanadyl ion). These complexes typically have square pyramidal or bipyramidal geometries with an axial oxo ligand [138]. The reduced form of V(IV) ($VOSO_4$) is blue in colour and has peak of absorbance at about 750 nm in the visible spectrum and shifts its peak to about 600-650 nm at neutral pH. Vanadyl interacts readily with carbonate [9,41], phosphates [9,139,140], pyridine, imidazole and other amine bases [9,139,140] and form different complexes. Hydrocarboxylic acid, phosphocarboxylate, nucleosides, nucleotides, catechols [9,139-142] etc. which contain more than one functionality form strong complexes with vanadyl cation [9]. These reactions are of physiological interest. Interaction of vanadyl with cysteine, cystene[38], picolinic acid [40], N, N-ethylenediamine diacetic acid [143] etc. forms complexes which possess promising insulin-mimetic properties. Bis(maltolato)oxovanadium(IV) (BMOV) is a compound recently developed for oral treatment of diabetes mellitus [10,134].

The co-ordination chemistry of V(V) compounds is dominated by oxo complexes containing VO^{3+} and VO_2^+ oxycations. The ^{51}V-NMR spectrum of a solution of vanadate at neutral pH will normally reveal at least four different peaks [144]. These correspond to OVO^+,

VO_4^{3-}, $HVO_4^{2-}(V_1)$ and $H_2VO_4^{-}(V_1)$ which result from a series of complex, rapid hydrolysis and polymerization reactions which are concentration and pH dependent.

Vanadium(V) comfortably binds different functionalities including O, N, S and form number of complexes with many organic and inorganic ligands having different co-ordination geometries[9]. Vanadium is stereochemically flexible with coordination geometries ranging from tetrahedral and octahedral to trigonal pyramidal and pentagonal bipyramidal being thermodynamically plausible[145]. Thus vanadate is a very labile system which rapidly interacts with a variety of naturally occurring organic compounds such as carboxylates, catechols, phenolics, neucleoside derivatives, amines, amino acids, peptides and proteins[144,146]

The chemistry of tungsten coordination compounds is exceptionally complicated[15]. The reasons are: (i) Tungsten forms complexes in oxidation states ranging between -2 to +6. (ii) The coordination number is variable which can go up to maximum of 13. (iii) Tungsten has the tendency to form clusters and polynuclear complexes[15] with varying number of atoms. In these compounds the tungsten-to-tungsten bond varies between single and quadruple. The lower the valence state the higher the degree of W-W bonding.

The possible donor atoms and the corresponding ligands encountered in tungsten compounds are presented in Table1.3. Biologically significant oxidation states of tungsten are +4, +5 and +6[15,26,93]. Hexavalent tungsten can be found in the form of hexahalides, which give rise to a variety of substitution products containing the structural unit W^{6+}. Hexavalent tungsten in addition shows a strong tendency to form bonds of higher order than one with donor atoms like O, S, Se or N. Consequently, distinction has to be made between complexes of the structural units W^{6+}, WO^{4+} and WO_2^{2+} and the analogous complexes which contain =S,

=Se or =NR instead of oxygen[15]. Thiotungstate ions like WS_4^{2-}, WOS_3^{2-} and $WO_2S_2^{2-}$ act as bidentate ligands for other metal ions. A prominent feature of the chemistry of tungsten is the formation of polytungstate (VI) acids and their salts. Vanadium (V) shows comparable behaviour but to a more limited extent. The polyacids are of two types: (i) isopolyacids[147,148], which contain only tungsten along with oxygen and hydrogen and (ii) heteropolyacids[149], which contain one or two atoms of another element in addition to tungsten, oxygen and hydrogen[150,151].

Table 1.3 *Donor atoms and corresponding ions or groups in tungsten coordination compounds*[15].

Donor atom	Ion/ group
C	Aldehyde, carbonyl, isocyanide, cyanide
N	Nitride, amine, thiocyanate, nitrile, pyridine, dinitrogen
P	Phosphine derivatives
As	Arsine derivatives
O	Aqua, oxo, peroxo, alkoxide, aryloxide, carboxylate
S	sulfides, persulfide, thiolato, dithiol, sulfate
Se	Selonide
F	Floride
Cl	Chloride
Br	Bromide

In pentavalent tungsten complexes the units W^{5+} and WO^{3+} also exist, but in addition dimeric structures can be found containing the $W_2O_4^{2+}$ unit[15]. Tetravalent tungsten complexes contain the structural units W^{4+}, WO^{2+} or dimeric configuration having W=W double bonds, as well as trinuclear clusters with three W atoms bonded together in a

triangular configuration. Most complexes of trivalent tungsten are dinuclear such as in the $[W_2Cl_4]_n^{3-}$ ion with some of them having a triple bond between the two tungsten atoms.

All known tungstoenzymes have two pyranopterindithiolate ligands [26,73,93,152]. Chemical and EXAFS studies suggest that WdO, WdS, and/or WsSH groups may also be present[153-155]. Interest in modeling the enzymes continued unabated[156] since the findings that tungsten-containing oxotransferase and hydroxylase enzymes posses a universal pterin dithiolene cofactor[26,73,157-159] above. Several synthetic chemical analogues of tungstoenzymes with these terminal functions have been prepared and investigated[73,93,152,160]. A dioxodithiolato- tungsten complex $[Net_4]_2[W^{VI}O_2\ (ndt)_2]$. H_2O (ndt = 2,3-naphthalenedithiolate) has been synthesized and structurally characterized [161,162]. These developments have added new significance to the chemistry of the element.

One of the most interesting aspects of tungsten and vanadium chemistry, which has also engaged the attention of several groups of contemporary researchers, is their peroxo chemistry[46,65,115,117-122,163-168] Peroxo-transition metal complexes in general have received continued attention over several years because of their important roles in biological processes[169-171] and in catalytic oxidations[171-180].

1.4 GENERAL FEATURES OF METAL-DIOXYGEN COMPLEXES

Molecular oxygen functions both as a ligand and as a reagent in transition metal chemistry. Recent interest in the chemistry of molecular oxygen has involved biochemists interested in biological oxygen transport and oxygen function[169,170,181-183] as well as industrial

chemists interested in developing homogeneous analogues to heterogeneous metal-catalysed oxidation reactions[114-116,172-180]. The isolation and characterization of stable dioxygen complexes and the variety of reactions that they themselves undergo are beginning to yield general information about bonding, structure, and reactivity of co-ordinated molecular oxygen [115,184-187].

Although the term molecular oxygen refers only to the free uncoordinated O_2 molecule with the ground state configuration $^3\Sigma g$, the term dioxygen has been used as a generic designation for O_2 moiety in any of its several forms and can be referred to O_2 in either a free or combined state[188]. For use of this term it is essential that a covalent bond exist between the oxygen atoms. Thus a metal dioxygen complex refers to a metal containing O_2 group co-ordinated to the metal center, and no distinction is made between neutral dioxygen in any of its reduced forms.

MO theory predicts bond orders of 2.5, 2, 1.5, and 1 for the dioxygenyl cation O_2^+, molecular oxygen O_2, superoxide O^{2-}, and peroxide O_2^{2-} are summarized in Table 1.4.

Table 1.4 *Physical data for dioxygen species* [171]

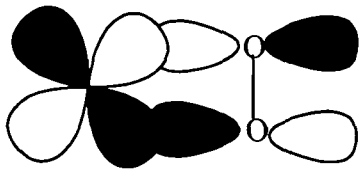
Dioxygen species	Bond order	O-O (A°)	Number of π^*_{u2p} electrons	ν(O-O) cm^{-1}
O_2^+	2.5	1.12	1	1858
O_2	2	1.2074	2	1556, 1554.7
O_2^-	1.5	1.32-1.35	3	1145 (KO_2)
O_2^{2-}	1	1.48-1.49	4	842 ($Na_2O_2.8H_2O$)

According to the rationalization made by Vaska[188], transition metal peroxides involve co-valently bound dioxygen resembling O_2^{2-} in peroxo configuration. A common characteristic of these complexes is the O-O distance, which occurs between 1.4 and 1.52 A° (1.49 for O_2^{2-}), and the corresponding infrared frequency ν(O-O), which lies between 800 and 950 cm^{-1}.

Simple peroxo compounds of transition metals are the ones, which contain peroxides, hydroperoxides and water molecules. Whereas heteroligand peroxo compounds, a term introduced by C. Djordjevic[170], refer to metal complexes containing one to three co-ordinated peroxo groups and one or more ancillary ligands. Heteroligands may range from monodentate ions to bulky porphyrins [5, 115,120,165,170,171,189](F^-, Cl^-, NH_3, SO_4^{2-}, $C_2O_4^{2-}$, CO_3^{2-}, NTA, EDTA, bipy, o-phen, oxine, porphyrins, pyridine-2,6-dicarboxylic acid etc.).

A comparison between the peroxo and unreduced dioxygen heteroligand complexes reflects that the chemistry of the two is very different owing to the presence of two extra electrons in the antibonding $O_{p\pi*}$ orbitals of the peroxide ion[170]. The electron rich O_2^{2-} ion therefore preferably forms complexes with metal ion of low d^n including d^0, and also f^0 electronic configurations, while the neutral dioxygen molecule favours higher d^n metal acceptors. However, there are at least two things that theses oxygen species have in common, viz., both are of importance to biochemistry[169,170,183].

The metal peroxo bonds in peroxo metallates are described by σ-interactions between the metal d_{xy} orbital and an in-plane peroxo π^* orbital as suggested from *ab initio* calculations and semi empirical computations[187] (*Fig. 1.1*). In case of diperoxo complexes the metal $d_{x^2-y^2}$ orbital interacts with π^* orbital of the second peroxo ligand to form the metal peroxo bond.



d_{xy} π^*

Fig. 1.1 Interaction of metal d_{xy} orbital with peroxo π^* orbital. Formation of metal-peroxo bond in peroxo metallates[187].

The way in which peroxo group is expected to co-ordinate to metals can range from symmetrical bidentate to a side-on monodentate position, including all possible angles in between them. The structural classification of dioxygen complexes, rationalized by Vaska [188] can be represented as shown in *Fig. 1.2.*

The bridging peroxo could vary from *cis*-planar and trans-planar to trans-nonplanar configuration. An unusual symmetrical double bridging was also found [190,191]. Deviations from the ideal symmetry are also observed very often[171,192,193]. In the cases of heteroligand fields they are due to the inherent symmetry of different donor atoms. Additional $p\pi^*$ electron delocalisation to the metal ion is anticipated, which could therefore favour d^0/f^0 or low d^n metal ion configuration. The stereochemical polyhedra in heteroligand peroxo complexes are often fairly predictable. In oxoperoxo heteroligand surrounding, the pentagonal bipyramidal

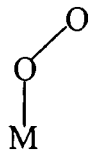
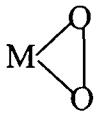
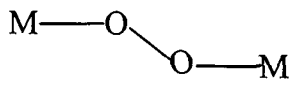
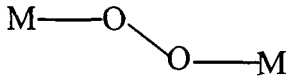
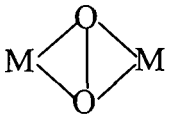
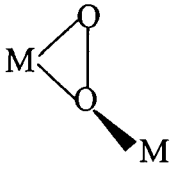
Structural type	Structural designation	Vaska classification
	η^1 dioxygen	Type a (superoxo)
	η^2 dioxygen	Type IIa (peroxo)
	η^1 : η^1 dioxygen	Type Ib (superoxo)
	η^1 : η^1 dioxygen	Type IIb (peroxo)
	η^2 : η^2 dioxygen	—
	η^1 : η^2 dioxygen	—

Fig. 1.2 Structural classification of metal-dioxygen complexes[188].

arrangement is most common for transition metal complexes, usually with two co-ordinated peroxo groups in *cis*- position.

Infrared spectroscopy is essential for the characterization of peroxo metal compounds. Coordination of peroxide in a side-on bidentate fashion creates a local C_{2v} symmetry which has three IR active modes[194], symmetric O-O stretching, symmetric metal-peroxo stretching, and antisymmetric metal-peroxo stretching which occur at approximately 880, 600 and 500 cm^{-1} respectively[115]. The ν_S(O-O) is the most sensitive and intense one. All

the three IR active modes are also Raman active and thus the results of Raman spectral studies not only complement the IR results but also augment them. Symmetric O-O stretching observed at approximately 850 cm^{-1} in IR is weak in case of bridging peroxide because of its very weak dipole, but it shows strong absorption in Raman spectroscopy.

The stability of peroxo complexes is generally enhanced by specific heteroligand combinations. Many simple metal peroxides often explode spontaneously, some are sensitive to shock or decompose above 0°C, several do not exist at all as stoichiometric compounds[103] but many heteroligand peroxo complexes, on the other hand, survive recrystallisation from boiling aqueous solutions, heating *in vacuo*, and remain unchanged for prolonged periods in closed containers[170,195-197]. The metals, Sc, Ti, V, Cr, Y, Zr, Nb, Mo, La, Hf, Ta, W[170] and U [198] form stable heteroligand peroxo complexes. The biochemical significance of peroxo metal complexes has been emphasized in literature[169,170]. The reactivity of peroxides[174,175,187,199-201] and the lability of metal-oxygen bonds in special heteroligand environments in solutions are of particular interest to biochemistry although not easy to measure directly.

1.5 PEROXO COMPOUNDS OF VANADIUM AND TUNGSTEN – CHEMISTRY AND IMPORTANCE

It has been known for over a century that characteristic colour reaction may take place when hydrogen peroxide is added to solutions of transition metal derivatives[184] and many peroxo transition-metal compounds have been isolated in the solid state[115,120,170,184]. There is currently considerable synthetic interest in the chemistry of peroxometallates[115,120,167]. Besides their scientific significance, such systems are attractive as

potential catalysts in biological[62-67,100,105] and industrial processes or their simple models[167,174,175,202-208]. Also, the research leading to gain an insight into roles of peroxo-transition metal complexes in storage and transport of oxygen and oxidase functions in biological systems is of growing interest[209,210].

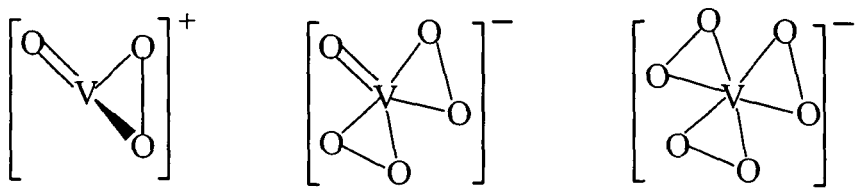
Among the various d^0 transition metal peroxo systems, V (V), Mo (VI) and W (VI) derivatives attract continuous growing research attention because of their versatility and selectivity as organic oxidants[115,120,174,175]. Knowledge regarding the active involvement of peroxovanadium compounds in haloperoxidases[128,211,212], their enzyme inhibitory[211], antineoplastic[32,33] and insulino-mimetic properties[5,6,10,36-46,61-64] as well as their potent catalytic properties in the oxidation of organic and inorganic substrates[115,174-176,202-208,212-214] have intensified interest in these complexes. Peroxo tungsten complexes, on the other hand, have been for the past several years object of investigation, mainly due to their application as an important class of stoichiometric or catalytic oxidizing and oxo-transfer agent in a variety of organic oxidations[117-122,215,216,217,218,219]. A perusal of literature however, shows that peroxotungsten chemistry has received relatively less attention compared to peroxomolybdates[120,220] in spite of the observation that pW compounds were more efficient oxidants compared to the Mo containing analogues[120,174].

There is a clear structural and isoelectronic relationship between peroxo complexes of Group 5 and Group 7 metals, which makes it worthwhile and convenient to study some of their features in parallel. Both vanadium as well as tungsten-hydrogen peroxide systems appear to be complicated owing to the formation of a number of different complexes in solution with a small change in pH of the reaction medium[167,184]. The composition of peroxo species of these metals formed in aqueous solution is sensitive to various factors viz., metal

and hydrogen peroxide concentration, pH, ionic strength, and reaction temperature [115,120,167,221].

Peroxotungstate(pW) and Peroxovanadates(pV) species formed in aqueous solution have been studied by several techniques including ^{17}O-NMR spectroscopy[187, 222,223], ^{183}W NMR[224], ^{51}V-NMR spectroscopy[201,222,225-228], Raman spectroscopy[225, 229] and by electrospray ionization mass spectrometry (ESI-MS)[213,230,231]. Moreover, structures of vanadium peroxo derivatives are also being theoretically investigated[213,231]. Because of the low receptivity of the ^{183}W neucleus, ^{183}W –NMR has been of limited use. However, Study of ^{51}V-NMR spectra of these compounds proved to be invaluable tool in identification of vanadium (V) reaction intermediates and compounds formed.

Monoperoxovanadate(MPV) species, $VO(O_2)^+$ appears at acid pH < 3.0 and low H_2O_2 : V(V) ratio and this imparts a red colour to the solution[167,184,232,233]. Diperoxovanadate(DPV) species, $[VO(O_2)_2(H_2O)_2]^-$ is formed in the broad pH range of 4.0-8.0 which is responsible for the yellow colour of the solution[141,174,234,235]. At higher peroxide and vanadium ratio and pH > 8.0 triperoxo[236] and tetraperoxo species dominate [103]. Most species have pH dependent ^{51}V-NMR chemical shifts arising from protonation and deprotonation reactions and were characterized by ^{51}V-NMR spectroscopy [222,225,228,232,233].



1 **2** **3**

Fig.1.3 Monomeric peroxo vanadium species. **1** monoperoxo; **2** diperoxo; **3** triperoxovanadate.

The notable points emerging out of the earlier studies[167,184,232,233,236] include the following:

(i) The number of peroxo groups per vanadium atom increases with alkalinity.

(ii) Increasing acidity increases polymerization and decreases the peroxy groups per vanadium atom.

(iii) Increasing concentration of H_2O_2 decreases the degree of polymerization.

In presence of molecules or ions with suitable donor atoms in the reaction mixture, the oxoperoxo ligand sphere tends to incorporate those molecules as ancillary ligands and thereby stabilizes the peroxovanadate moiety[5,115,170,189]. Thus, depending on the pH and reaction conditions monoperoxo, diperoxo or triperoxo complexes may be formed which are represented by the formulae: $M_n[VO(O_2)_2(L)]$, $M_n[VO(O_2)_2(LL')]$ or $M_n[VO(O_2)(H_2O)(LL'L'')]$ where M = NH_4, Na or K; n = 1-3; and L, LL′ and LL′L″ are mono, bi- and tridentate ancillary ligands[5]. A large number of peroxovanadium and oxodiperoxovanadium(V) complexes in diverse ligand environment have been structurally characterized and reported in recent years[5,115,165,189,237,238]. In general, peroxovanadate complexes are mononuclear with the vanadium atom in a pentagonal bipyramid with one or two peroxo groups bonded in a side-on fashion in the equatorial plane[5,115,165,189,237,238]. Dinuclear peroxovanadate compounds with various bridge configurations, although very limited, are known in which either an oxo group or donor atom of the heteroligand usually binds the two vanadium centers[165]. Djordjevic et al. have synthesized a series of oxo-bridged dimeric peroxovanadium complexes such as $(NH_4)_4[O\{VO(O_2)_2\}_2]$ and $M(I)_4[O\{VO(O_2)_2L\}_2]$, (L = cystine, adenine, adenosine) and observed that these dimers differ from the monomeric peroxo compounds tested in terms of solubility, stability towards decomposition and also toxicity and related properties of importance for medicinal

application[189]. A set of oxo-bridged peroxovanadates synthesized in our laboratory exhibited remarkable resistance to catalase[239,240]. Dinuclear peroxovanadate intermediates possessing a μ-peroxo bridge have been implicated in certain biochemical processes[241-244]. However, only a few reports regarding chemistry of such species of vanadium in solid state are available[190, 221, 245-249].

The reactivity of V(V) peroxo complexes, which is different from and much less selective than that of W-peroxo complexes, was tentatively attributed to a biradical V(IV) – O-O· species resulting from the hemolytic cleavage of the V(V) –oxo bond[220,250] . Various synthetic approaches have been developed for the oxidations of alkenes and allylic alcohols to corresponding epoxides[115,174,175,213,250,] primary and secondary alcohols to the aldehydes and ketones[115,213,251,252], aldehydes to esters [253,254], sulfides to sulfoxides and sulfones[115,176,178,213] as well as, hydroxylations of alkanes and arenes[115,174,213,250 ,255](*Fig. 1.4*). The catalytic applications of peroxovanadates take advantage of the increased oxidation rate of peroxovanadium complex, which after formation reacts[256]. Owing to the biological significance of the peroxo-vanadate mediated oxidations, most of the recent studies have been conducted in water [213 ,257,258]. However, peroxovanadate compounds are also efficient oxidizing agents in less polar organic solvents [175,250,259]. Recent developments have been utilizing biphasic, phase transfer systems as well [260,261].

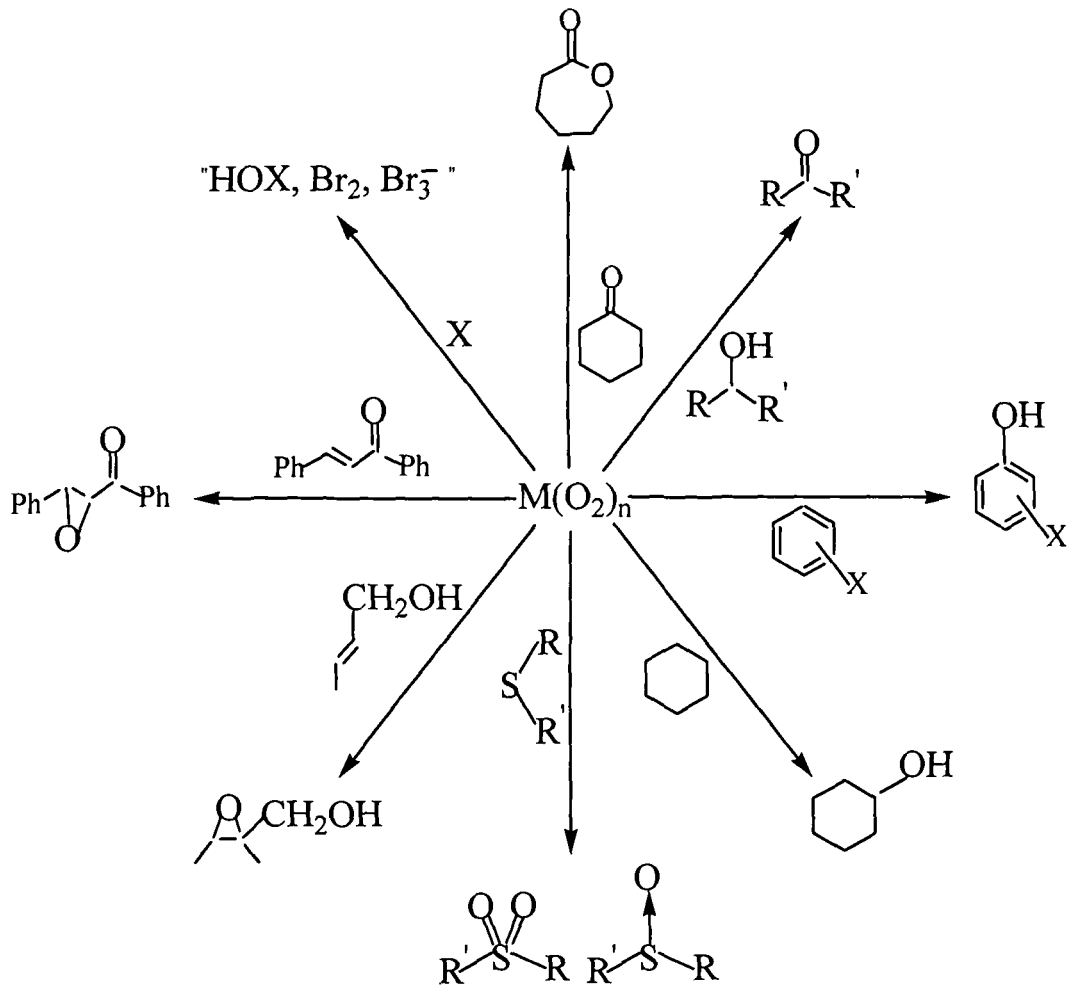


Fig. 1.4 Selected oxidations of organic compounds by peroxometal complexes.(M=V,W)[260]

The tetraperoxotungstate anion $[W(O_2)_4]^{2-}$ is the principal species in an alkaline solution (pH 7-9) of WO_4^{2-} and excess H_2O_2. Several salts of the yellow coloured anion have been reported[184] all of which are unstable and prone to explode. As the pH of peroxide-rich

solutions of tungstate is lowered to <5 tetraperoxoanions are converted into dimers of diperoxo species[120].

$$2[W(O_2)_4]^{2-} + 5H_2O \rightleftharpoons [M_2O_3(O_2)_4(H_2O)_2]^{2-} + 2OH^- + 4H_2O_2$$

A number of salts of the oxo-bridged tetraperoxoditungstate complex anion viz., $[W_2O_3(O_2)_4]^{2-}$ has been isolated and structurally characterized[120]. However, there appears to be a paucity of information on synthesis of complexes with $[W_2O_3(O_2)_4]^{2-}$ moiety stabilized by a heteroligand other than H_2O [120-122,262,263]

Formation of the monomeric diperoxotungsten species $[WO(O_2)_2(H_2O)_2]$ is favoured in dilute (*ca.* 0.1 mM) acidic solution (0.10-1.00 M, H^+)[264,265]. Several oxodiperoxotungsten(VI) complexes in different heteroligand environment have been reported[70,71,120-122,234,235,262,266,267] and in some cases structurally characterized (Table 1.5). In all the structurally characterized complexes of the type, $[MO(O_2)_2L_{ax}L_{eq}]$,[0/1-/2-] the metal atoms are seven coordinated, with a pentagonal bipyramidal geometry[120]. The oxo and the two η^2-peroxo groups occupy mutually *cis* -positions with one L ligand in axial position and the remaining L being equatorial (Table 1.5). Ligands L may also function as bridging groups in dinuclear species.

Table 1.5 Some *structurally characterized monomeric and dimeric oxodiperoxo complexes of tungsten(VI)*[120]

Stoichiometry	L_{ax}	Leq	n	Ref.
$[WO(O_2)L_{ax}(Leq)_3]^{n-}$	F^-	F^-	2-	268
$[WO(O_2)_2L_{ax}L_{eq}]^{0/1-/2-}$	H_2O	hmpt	0	269
	H_2O	$(tacn)WO_3$	0	270
	½(ox)	½(ox)	2-	266
$[WO(O_2)_2L_{ax}L_{eq}]_2^{2-}$	H_2O	H_2O	2-	271
	¼(μ_4-ox)	¼(μ_4-ox)		

The pertungstate- hydrogen peroxide mixture has been used for oxidation of a variety of alkenes such as isolated double bonds[272,273] allylic and homoallylic alcohols [274] and α,β-unsaturated acids[117,275] and are considered to be the best transition metal catalyst for epoxidation reactions of alkenes with hydrogen peroxide The combination of WO_4^{2-} / H_2O_2 is used in industry for the preparation epichlorohydrin[208] the major raw material used in the manufacture of epoxy and phenoxy resins. Pertungstic acid and pertungstates are known to give highly stable aqueous solutions and the tungstate ion has been shown to be quite superior to molybdate and vanadate , since the transition metal ion induced decomposition of hydrogen peroxide is much slower and allows the use of a boarder pH range [276,277] of 6-7 .

The dimeric anions are believed to be the species responsible for tungstate catalysis of alcohol oxidation and alkene epoxidation by H_2O_2[120,225,278]. Organic solvent-soluble Ph_4P^+

and $Ph_3PhCH_2P^+$ salts of these species have been shown to be stoichiometric and catalytic epoxidants in dichloromethane and dichloroethane[120,225,272]. It is noteworthy that unlike the neutral diperoxo complexes or oxo-bridged dimeric complexes, the dianions were ineffective at epoxidation of alkenes[120,278].

In 1969 Mimoun et al.[279] introduced an important class of neutral seven-coordinated peroxo complexes of Mo and W, $[MO(O_2)_2Lx]$ (L = py, hmpa, dmf, H_2O and so on; x =1,2), as an important class of stoichiometric oxidants for organic oxidations. Because of their higher solubility in polar and non polar solvents, the neutral and appropriate salts of mono anions of peroxo tungsten complexes have been widely used as both stoichiometric and catalytic oxidants in oxygen-transfer reactions including oxidation of primary and secondary alcohols to aldehydes and ketones respectively[122,280,281,] epoxidation of alkenes[215,269] of sulphides and sulphoxides to sulphoxides and sulphones[282,283] , indoles, furans, organoboranes, metal alkyls (*Fig.1.4*) Although in majority of these investigations $[MoO(O_2)_2(hmpt)]$ has been the complex of choice but studies indicate that the diperoxo species $WO(O_2)_2HMPA$ is a more effective oxidant than $MoO(O_2)_2HMPA$ in alkene epoxidation[120,220].

A significant contribution to the existing wealth of organic oxidants is the 'Venturello's complex' $(R_4N)_3[(PO_4)W_4O_4(O_2)_8]$ isolated and characterized structurally by Venturello and co-workers[216]. Such complexes, with H_2O_2 as co-oxidant, catalyze the oxidations of a wide variety of organic substrates; normally in biphasic solvent systems with phase transfer agents. The reactivity of the species is believed to result from the presence of pairs of bridging peroxo ligands[216]. Tungsten catalysts of this type use H_2O_2 more efficiently

than many other epoxidation catalysts; insofar as their unique chemistry favours oxygen transfer over peroxide disproportionation[276,277].

Quite exciting is to draw attention to an important development in the area of green chemistry is the work of R. Noyori[217]. In continuation of his design of routes to greener synthesis Noyori described oxidation of various organic compounds using H_2O_2, a physiologically harmless tungstate catalytic system consisting of Na_2WO_4 and $Q^+HSO_4^-$ (methyltrioctylammonium hydrogensulfate) as PTC (a phase transfer catalyst) without any organic solvents and halides[217,218,219,284].

The influence of ligation on the reactivity ofperoxide complexes of V(V), Mo(VI), (VI), and other transition metal ions is a topic of much interest. The nature of the coordinating ligand and the solvent system are very important factors on which the oxidative reactivity of peroxotungstate[260] and peroxovanadate complexes depend[285]. An increase of electron density on the metal brought in by the co-ligands would reduce the electrophilicity of peroxo complexes and also their ability to act as oxidant[201]. The activity of peroxotungsten and peroxovanadium complexes as catalysts have been fine-tuned with ligands and various correlations have been made involving the electronic and other properties of the ligand[186,201,256-258,285,286] The mechanism of oxidation reactions mediated by peroxotungstates and peroxovanadates as electrophilic or radical oxidants have been studied extensively[115,175,250,258].

Besides the oxidations of organic substrates, peroxo compounds of vanadium are also able to oxidize various inorganic substrates including sulfur dioxide[287], thiocyanate[288] and halides[115, 212] .The peroxo tungsten system has also been reported to catalyse the oxidation of bromide in acidic medium[289]. The oxidation of bromide with peroxo -metal systems is of

particular interest as such a process is actually a chemical model of the activity of vanadium-dependent haloperoxidases[115,128].

Haloperoxidases are enzymes that catalyze the two-electron oxidation of halide (X^-) by peroxide to the corresponding halogenating species X_2, X_3^- or hypohalous acid, which halogenates organic substrates RH [13,53, 115,128, 135,290].

$$RH + HX + H_2O_2 \xrightarrow{V\text{-}HPO} RX + 2H_2O$$

The primary oxidized intermediate is still not known although for bromide it is equivalent of hypobromous acid, bromine, tribromide or an enzyme-bound bromonium ion-type species[13,53,115,128,135,290]. They are referred to as chloroperoxidases, bromoperoxidases or iodoperoxidases depending on the most electronegative halogen they can oxidize.

Bromoperoxidases, are involved in the biosynthesis of many brominated marine natural products ranging from simple hydrocarbons to halogenated terpenes, indoles, phenols, which often have important biological and pharmacological activity[291]. In absence of an organic halogen acceptor, the oxidized bromine reacts with a second equivalent of hydrogen peroxide resulting in the formation of bromide and singlet oxygen[115,291].The disproportionation reaction of hydrogen peroxide is a bromide-catalyzed process. In addition to halide oxidation, the vanadium haloperoxidases and some of their model compounds are capable of oxidizing organic sulfides to sulfoxides[115,176,177,213].

Crystal structures of some haloperoxidase proteins *Curvularis inequalis*[292], *Ascophyllum nodosum*[293], *Corallina officinalis*[294] are now available. In the native site a five co-ordinated trigonal-bipyramidal vanadium (V) moiety is bound to three non-protein oxo

groups in the equatorial plane and one histidine and hydroxy group at the axial positions, the architecture being similar to evolutionary-related acid phosphtase[295]. The oxygens are hydrogen bonded to several amino acid residues of the protein chain. Addition of peroxide converts the arrangement from trigonal-bipyramidal to tetragonal pyramidal with the peroxo ligand in the tetragonal plane and oxo-oxygen in the apical position. Quite interestingly, bromoperoxidase show phosphatase activity after removal of vanadate[55] and the peroxidase activity can be restored on reconstitution of the apoenzyme with vanadate.

The vanadium-dependent bromoperoxidase is now the subject attracting much attention of chemist as well as biologist. Studies on synthetic models of V-BPO have been extremely useful in helping to unravel details of the structure and mechanism of activity of the enzyme. The biomimetic functional models reported in the literature [56,125,128-130,241,242,291] are mostly based on monoperoxovanadium[125] or triperoxodivanadium species[56,128,129,241,242,291].Schieff-base complexes of V(V), aqueous solution of cis-dioxovanadium(V) (VO_2^+) in acidic medium, a V_2O_5 and H_2O_2 system, KBr in excess H_2O_2 in presence of vanadyl sulphate in phosphate buffer were all found to be effective in bromination of organic substrates and were studied in detail as functional mimic of the enzyme. Apart from vanadate and peroxovanadate, a few other transition metal systems such as MoO_3(aq)[289],WO_3(aq)[289],$MeReO_3$[296], MoO(oxalate)[297] catalyze the oxidation of bromide by hydrogen peroxide and are thus treated as functional mimics of V-BPO[298]. A tungstate-exchanged layered double hydroxide has also been studied as a heterogeneous catalyst in oxidative bromination of olefines by H_2O_2 system[180,299].

Concomitant with the biochemical interest on the activity of V-BPO there have been efforts to develop catalytic protocols with synthetic V-BPO mimics [179,300,301]. Conventional

bromination methods involve elemental bromine, which is a pollutant and a health hazard. There is a need for benign catalytic systems, which can mimic the biological bromoperoxidase in the synthesis of brominated organics[300]. Catalytic protocols with most V-BPO biomimics still contain major disadvantages, such as the use of chlorinated solvents. Moreover, unlike V-BPO which functions most efficiently at near neutral pH[115,124] most of the model complexes were found to be catalytically active only in acid medium. It is worth mentioning here that, success has been achieved by other workers of the laboratory where the present work has been carried out, in synthesizing a series of pV compounds with the distinctive feature of having a μ -peroxo group of the type $[V_2O_2(O_2)_3.(L)_3].H_2O$ (L = amino acid or dipeptide)[248,] which could act as powerful oxidant of bromide with good activity at physiological pH thus mimicking the enzyme V-BPO. Diperoxovanadate compounds with exclusively peroxo groups in its co-ordination sphere were found to be catalytically incompetent in bromide oxidation at neutral pH[221]. The μ-peroxo V compounds however, undergo rapid degradation in solution with loss of its high bromination activity[221].

Thus from the foregoing discussion it is evident that despite the progress made in gaining an insight into the various aspects of activity of V-BPO, the exact mechanistic details of the enzyme function is yet to be fully understood and hence is still a subject of study. Also a great deal of effort is still required to develop peroxometallates with definite potential for application as safer alternative synthetic catalyst for organic bromination reactions. Much remains yet to be explored on activity of well-defined pW compounds in oxidative bromination .

Other very important aspects of pV systems of contemporary interest are their antineoplastic[32,34] and insulin mimetic effects[5,6,10,36-40,43-46,61-64]. Djordjevic et al. tested a

range of heteroligand pV compounds for their anti tumor activity and observed that such activity was dependent on the nature of the hetero-ligand and the cation present[34]. According to recent reports, diperoxovanadate complexes were found to be effective as drug for the treatment of infectious diseases, in immune disorders or infections caused by HIV virus[302].

Vanadate and peroxide have been known to act synergistically to mimic insulin activity[303]. Several stable peroxo complexes of vanadium having the general formula $A_n[VO(O_2)_xL\text{-}L']$.$yH_2O$ where A^+ is NH_4^+ or K^+, n is 0-3, x is 1 or 2 and L-L$'$ is usually a bidentate ligand were found to be effective insulin mimics by Shaver et al.[5]. Apart from peroxovanadates, it has now been established that peroxotungstate and peroxomolybdate also stimulate most of the insulin bioeffects in rat adipocytes[100]. Moreover, pW and pMo were found to be 80-180-fold more potent stimulators than the corresponding metalloxides[100]. The mechanism by which these peroxometallates mimic the action of insulin is not completely understood[100,109-111]. A correlation has been found to exist between the inhibitory effect of vanadate and pV complexes on protein phosphatases, and their *in vivo* insulin mimetic activities[109,110-112] . It has been proposed that pV complexes bind to the active site of phosphatases because they are quite similar to phosphate ester and inhibit the enzyme by irreversibly oxidizing the cystein residue in the active site of the enzyme[5]. The insulin mimetic complexes $K_2[VO(O_2)_2pic].2H_2O$ and $K_2[VO(O_2)_2(OHpic)].3H_2O$ were indeed capable of oxidizing cystein to cystine[115]. Shechter and co-workers observed[100] that the higher efficacy of the pertungstates and permolybdates as insulinomimetic agents originated from their oxidizing activity relative to GSH.

A large number of heteroligand pV complexes have been tested for possible insulin like activities in recent years[304]. However, most of the peroxo compounds are hydrolytically

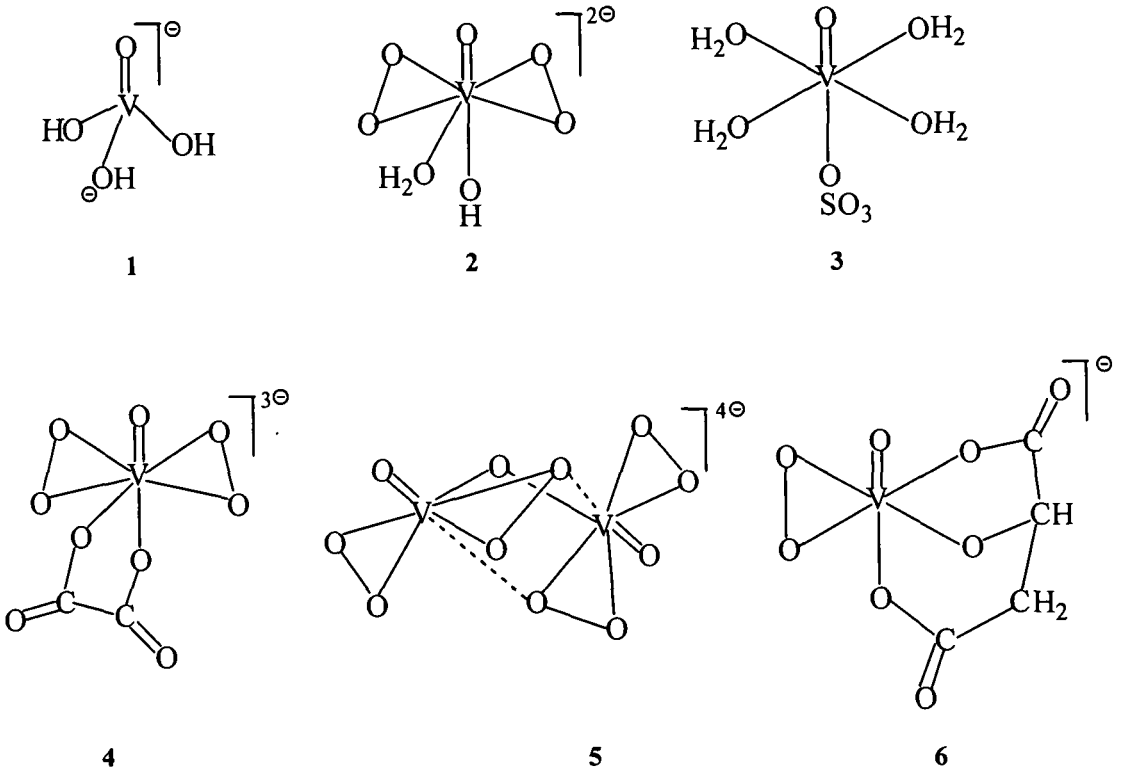


Fig. 1.5 Vanadium compounds of therapeutic importance. These compounds have already been proved to be active in animal tests[7].

unstable and end with radical formation when subjected to redox processes[63,305] which limits their utility as therapeutic agents. Thus there is an urgent need for stable, better absorbed, more efficacious vanadium compounds with therapeutic potential and this has spurred an intense search for biologically relevant peroxo-vanadium complexes. On the other hand, inspite the observation that peroxotungstates, formed in a solution of $W\text{-}H_2O_2$ were stable in solution of a wide range of pH values[100], there appears to be no information available pertaining to studies involving the effect of well-defined synthetic heteroligand peroxotungsten compounds on activity of relevant or their insulin mimetic properties.

1.6 REASEARCH OBJECTIVES

It may be inferred from the above non-exhaustive discussion, that the peroxo chemistry of d^0-transition metals viz., vanadium (V) and tungsten (VI) in general embraces a fascinating, rewarding and worthwhile area of investigation. Also it is evident that the synthesis of well defined peroxo complexes of these metals and assessment of their structures, stability and redox properties are above all prerequisites following which other aspects can be developed.

Major objectives of the present research programme are as follows:

(i) To synthesize newer stable peroxo complexes of tungsten and vanadium stabilized by suitable co-ligands of physiological relevance and to characterize them. It is of particular interest to obtain dinuclear heteroligand compounds of tungsten possessing bridging oxo group with a $[W_2O_3(O_2)_4]^{2-}$ moiety.

(ii) To study the stability of the compounds towards decomposition in the solid state as well as in solution.

(iii) To investigate the oxidant activity of the compounds synthesized, particularly the peroxotungstates, in bromide oxidation and oxidative bromination of organic substrates with an aim to pursue biomimetic chemistry of bromoperoxidase.

(iv) To undertake investigations involving studies on the interaction of the pV and pW compounds as substrates for enzyme catalase, their oxidant activity with

respect to reduced glutathione, and the possible inhibitory effect of the pV and pW compounds on the alkaline phosphatase activity.

Chapters **3** to **6** of the thesis present interpretative accounts of the results of our studies on the afore mentioned aspects of peroxotungsten and peroxovanadium chemistry. Each of these Chapters has been so designed as to make it a self-contained one with brief introduction, sections on experimental, and results and discussion followed by relevant bibliography. In Chapter **7** some general conclusions drawn from the results of the work undertaken have been presented. The results have been either published or accepted for publication.

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CHAPTER 2

2.1 CHEMICALS

The chemicals used were all reagent grade products. The sources of chemicals are given below:

Sodium and potassium tungstates, amino acids, sodium and potassium vanadates, cysteine, sodium thiosulphate, potassium persulphate (CDH); Vanadium pentoxide and vanadyl sulfate (SRL); hydrogen peroxide 30% (v/v), potassium bromide, potassium iodide, potassium hydrogen phosphates (E. Merck, India); Tungstic acid, dithionitrobenzoic acid (DTNB), glutathione (GSH) (Himedia laboratories, Mumbai,India); Alkaline phosphatase from rabbit intestine, p-nitrophenyl phosphate (p-NPP), glycyl-peptides, phenol red, catalase, (Sigma Alderich Chemicals Company Pvt. Ltd.); Potassium dihydrogen phosphates, sodium and potassium hydroxides, aniline, nitroanilines, aminophenols, quinol, 2-methoxytoluene, acetone, diethyl ether, ethyl acetate, petroleum ether 40°-60°C (SD fine chemicals). Sodium diperoxovanadate (NaDPV) was prepared by the method described earlier[1].

Solutions were made fresh before the experiments in water, doubly distilled in a quartz apparatus after initially passing through milli RO water purification system.

2.2 ELEMENTAL ANALYSES

2.2.1 Tungsten[2]

Tungsten was estimated gravimetrically as $BaWO_4$[2]. An accurately weighed amount

of the compound was taken in a beaker containing 250 ml of distilled water. pH of the solution was adjusted to *ca.* 7 by adding NaOH (0.1M) and was boiled for *ca.* 30 min. To the boiling solution a saturated $BaCl_2$ solution was added dropwise with constant stirring. The precipitate formed was allowed to settle for a few minutes. The supernatant liquid was tested for complete precipitation by adding few drops of barium chloride solution. A slight excess of precipitating agent was added to ensure complete precipitation. The mixture was kept covered over a steam-bath for 1hr in order to allow time for complete precipitation of $BaSO_4$. The precipitate was then allowed to settle at room temperature and the clear supernatant liquid was again tested for complete precipitation. The digested precipitate was then filtered through a constant-weighed sintered glass crucible (Grade 4) and ignited at 500°C in an electric muffle furnace followed by cooling in a desiccator. The heating process was continued until constant weight was obtained.

2.2.2 Vanadium[3]

Vanadium was estimated volumetrically by titration with a standard potassium permanganate solution[3]. A near boiling solution of an accurately weighed amount of the vanadium(V) compound, after removing peroxide, was treated with a stream of sulphur dioxide for *ca.*10 min, and then with a stream of carbon dioxide to expel any excess of sulphur dioxide. The vanadium (IV) solution thus obtained was cooled at a*c.* 80°C, and finally titrated with a standard potassium permanganate solution.

2.2.3 Peroxide[4-6]

2.2.3.1 Permanganometry[4]

An accurately weighed amount of a peroxovanadate compound was dissolved in 7N sulphuric acid containing *ca.* 4 g of boric acid. Boric acid was used to form perboric acid to prevent any loss of active oxygen. The resulting solution was then titrated with a standard potassium permanganate solution.

1 ml of 1N $KMnO_4$= 0.01701 g of H_2O_2

This method is suitable for determination of peroxide content in peroxovanadium(V) compounds.

2.2.3.2 Iodometry[5]

To a freshly prepared 2N sulphuric acid solution, containing an appropriate amount of potassium iodide (~1 g in 100 ml) was added an accurately weighed amount of peroxovanadate(V) compound with stirring. The mixture was allowed to stand for *ca.* 15 min in CO_2 atmosphere in the dark. The amount of iodine liberated was then titrated with a standard sodium thiosulphate solution, adding 2 ml of freshly prepared starch solution, when the color of the iodine was nearly discharged.

1 ml of 1N $Na_2S_2O_3$ = 0.01701 g of H_2O_2

This method gives the total amount of peroxide plus vanadium present in the compound. On deduction of the contribution of vanadium(V) from the total amount of iodine liberated, the net peroxide content of the compound is evaluated.

2.2.3.3 By standard Ce(IV) solution[6]

An accurately weighed amount of a peroxotungstate(VI) or a peroxovanadate(V) compound was dissolved in a 2N sulphuric acid solution in the presence of an excess of boric acid. Peroxide was then determined by titrating with a standard Ce(IV) solution. Tungsten(VI) and vanadium(V) does not interfere in this method.

2.2.4 Carbon, Hydrogen and Nitrogen

The compounds were analyzed for carbon, hydrogen and nitrogen by micro-analytical methods at the Regional Sophisticated Instruments Center (RSIC), North-Eastern Hill University, Shillong, India and at the Department of Organic Chemistry, Indian Institute of Science, Bangalore, India.

2.2.5 Sodium and Potassium

Sodium and potassium contents were determined by Atomic Absorption Spectroscopy.

2.3 PHYSICAL AND SPECTROSCOPIC MEASUREMENTS

2.3.1 pH measurement

pH of the reaction solutions, whenever required were measured by using a Systronics μ pH system 361, and also by E. Merck Univrsalindikator pH 0-14 paper.

2.3.2 Molar conductance

Molar conductance measurements were made at ambient temperature using Systronics Conductivity Meter 306.

2.3.3 Magnetic susceptibility

Magnetic susceptibilities of the complexes were measured by the Gouy Method, using Hg $[Co(NCS)_4]$ as the calibrant.

2.3.4 Electronic spectra

Spectra in the visible and ultraviolet regions were recorded in a in a Cary model Bio 100 spectrophotometer, equipped with a peltier controlled constant temperature cell and also in a Shimadzu double-beam UV 160 A or a Hitachi model 2001 recording spectrophotometer in 1-cm quartz cuvettes. All the absorbance values are denoted as, e.g., A_{592}, A_{340} at the wavelengths indicated.

2.3.5 Infrared (IR) spectra

The infrared (IR) spectra were recorded with samples as KBr pellets in a Nicolet model impact 410 FTIR spectrophotometer and also in a Perkin Elmer Model 983 spectrophotometer.

2.3.6 ^{1}H-NMR spectra

The ^{1}H-NMR spectra were recorded in deuterated chloroform either in Varian EM-390 90 MHz NMR spectrophotometer or Varian T-60 instrument. TMS was used as an internal standard. Values are given in ppm ; s, d, m and br are used to depict the singlet, doublet, multiplet and broad absorption signals respectively in ^{1}H-NMR spectrum.

2.3.7 ^{51}V-NMR spectra

The ^{51}V-NMR spectra were recorded in a Brucker AMX 400 FT spectrometer at vanadium frequency 105.190 MHz with the samples in a 10 mm spinning tube with a sealed coaxial tube containing D_2O to provide the lock signal. The chemical shift data are shown as negative values of ppm with reference to $VOCl_3$ at 293 K.

2.3.8 HPLC analysis

HPLC analyses were performed using a Waters Tm 2487 dual λ detector and assayed at fixed wavelengths using C_{18} column (Nova-Pak C_{18}, 3.9 × 150 mm, Waters).

2.3.9 Thermogravimetric analysis

Thermo gravimetric analysis was done in Mettler Toledo Star system at a heating rate of 5 ^{0}C/min under the atmosphere of nitrogen using aluminum pan.

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CHAPTER 3

New mononuclear and dinuclear peroxotungsten(VI) complexes containing biogenic co-ligands. Synthesis, characterization and stability*

3.1 INTRODUCTION

The importance of and the interest in peroxotungsten (pW) compounds, which rendered them the focus of one of the active areas of contemporary research, have been emphasized in the literature[1-4] and highlighted in the introductory Chapter. Our interest in the design, synthesis and study of pW compounds has been spurred by the importance of pW systems mainly attributable to their catalytic potential[1,5,6], as well as the increasing awareness of the biochemical relevance of tungsten and its compounds[7-9].

A large number of heteroligand peroxo complexes of tungsten in diverse co-ligand environment has been synthesized and characterized[1,10-14], a majority of which are diperoxo tungsten species isolated as neutral, monoanionic or dianionic complexes. Previous studies indicate that while the neutral diperoxo complexes could act as efficient oxidants, the dianions were catalytically incompetent in epoxidation of alkenes[1,11]. The oxo-bridged dimeric tetraperoxo species $[W_2O_3(O_2)_4(H_2O)2]^{2-}$, have been known to exist in a solution of tungstate and excess peroxide at pH $\leq$ 5 and are believed to be the species responsible for tungstate catalyzed oxidations by H_2O_2 [1,10,11]. A few salts of the

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structurally characterized oxo-bridged complex species were documented to be efficient catalytic epoxidants[1,10,11]. In spite of these important findings, to the best of our knowledge, synthesis of complexes with oxo-bridged dimer of diperoxo tungsten, $[\{WO(O_2)_2\}_2O]^{2-}$moiety stabilized by a heteroligand other than H_2O has not been reported till date[1,12]. Moreover, although many reports deal with the synthesis and reactivity of heteroligand peroxotungsten compounds[1,11-14], information on synthetic peroxo-W(VI) complexes with co-ordinated biogenic ligands are scanty[1,12].

In view of the above observations in the present work we endeavored to establish rational synthetic routes to new oxo-bridged dimeric and monomeric peroxo-tungsten complexes containing amino acid or dipeptide as co-ligand, with appropriate characteristics of solubility and stability. Our primary goal has been to gain an access to peroxo tungsten complexes with biologically relevant properties, which may also be useful as oxidant in organic oxidation including oxidative bromination. In addition, it was considered imperative to investigate the possible similarities or differences in stability, redox or solution properties, which may exist between monomeric and dimeric peroxotungsten complexes in analogous co-ligand environment. In view of the importance of cysteine, cystine in biological redox processes we were particularly interested to study the reaction of $W\text{-}H_2O_2$ with this ligand and expected that a dinuclear tungsten system with a S-containing ligand in its co-ordination sphere would constitute an interesting combination. A planning of synthetic strategies and working out of appropriate experimental conditions are important prerequisites for the synthesis of new well-defined peroxo metal derivatives.

The present Chapter reports the synthesis and characterization of hitherto unreported dinuclear peroxo complexes of tungsten containing amino acid or dipeptides as co-ligands of the type, $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$ [A = Na (**3.1**) or K (**3.2**)], $Na_2[W_2O_3(O_2)_4(dipeptide)_2].3H_2O$ [dipeptide = glycyl-glycine (**3.3**) and glycyl-leucine, (**3.4**)] and neutral monomeric compounds, $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$ [dipeptide = glycyl-glycine (**3.5**) or glycyl-leucine (**3.6**)] are presented. Also reported in this chapter are the results of studies on the stability of the compounds towards decomposition in solution.

3.2 EXPERIMENTAL SECTION

3.2.1 Synthesis of dinuclear peroxotungstate complexes, $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$ [A = Na(3.1) or K (3.2)]

As the methods of synthesis of the compounds **3.1** and **3.2** are similar, a representative procedure is described.

A 0.5 g (1.5 mmol) sample of A_2WO_4 was added to a solution of cysteine (0.24 g, 1.5 mmol) in 5 ml of water. Keeping this mixture in an ice bath 5 ml of 30% H_2O_2 (44.1 mM) was added gradually with constant stirring until all solid dissolved and a clear colorless solution was obtained. The pH of the solution was recorded to be 2.5. No attempt was made to adjust the pH of the reaction solution. On adding pre-cooled acetone (*ca.* 50 ml) to the above solution under continuous stirring, a white pasty mass separated out. After allowing it to stand for about 10 min in the ice bath, the supernatant liquid was

decanted and the residue was treated repeatedly with acetone under scratching until it became a microcrystalline solid. The product was separated by centrifugation, washed several times with cold acetone and finally dried *in vacuo* over concentrated sulfuric acid.

3.2.2 Synthesis of dinuclear peroxotungsten complexes, $Na_2[W_2O_3(O_2)_4(dipeptide)_2].3H_2O$ [dipeptide =glycyl-glycine (3.3) and glycyl-leucine, (3.4)]

The procedure adopted for the synthesis is common to both complexes. This consisted of a slow addition of 10 ml of 30% H_2O_2 (88.2 mmol) to H_2WO_4 (0.25 g, 0.87 mmol) contained in a 250 ml beaker The mixture was kept in an ice bath and stirred slowly until all the solids dissolved and a clear solution was obtained. To this solution dipeptide (0.87 mmol) was added. The pH of the clear solution was recorded to be *ca.*1.5. The pH of the reaction mixture was adjusted to *ca.* 2.5 by carefully adding powdered sodium hydroxide (46.0 mmol). Addition of pre-cooled acetone (*ca.* 50 ml) to the reaction solution obtained as above under continuous stirring, caused precipitation of a white pasty mass. After allowing it to stand for about 15 min in the ice bath, the supernatant liquid was decanted and the residue on being treated repeatedly with acetone under scratching yielded a microcrystalline solid. The product was separated by centrifugation, washed with cold acetone and dried under vacuum.

3.2.3 Synthesis of mononuclear peroxotungsten complexes, $[WO(O_2)_2(glyl\text{-}gly)(H_2O)].3H_2O$ (3.5) and $[WO(O_2)_2(glyl\text{-}leu)(H_2O)].3H_2O$ (3.6)

In a typical reaction 5 ml of H_2O_2 (30 % solution, 44 mmol) was added gradually to a mixture of H_2WO_4 (0.5 g, 1.7 mmol) and the respective peptide with continuous stirring, maintaining the W: dipeptide ratio as 1:1. Keeping the temperature below 4 ^{0}C in an ice bath, the mixture was stirred for *ca.* 5 min until all solids dissolved. At this stage the pH of the solution was recorded to be *ca.* 1.5. The pH of the reaction mixture was raised up to 5.5 by adding NaOH solution (0.1 M) dropwise. To the above reaction solution pre-cooled acetone (*ca.* 50 ml) was then added with continuous stirring. A colorless pasty mass separated out at this stage. The supernatant liquid was decanted off after allowing the mixture to stand for about 10 min in the ice bath. The residue upon repeated treatment with acetone under scratching gave a white microcrystalline solid. The product was separated by centrifugation, washed with cold acetone and dried *in vacuo* over concentrated sulfuric acid.

3.2.4 Elemental analysis

Quantitative estimations of tungsten, peroxide, carbon, hydrogen, nitrogen, sodium and potassium were accomplished by methods described in Chapter **2**. The analytical data of the compounds are summarized in Table 3.1.

3.2.5 Physical and spectroscopic measurements

Spectroscopic measurements, magnetic susceptibilities, molar conductances, TG analysis measurements were done as per methods described in Chapter **2**. Structurally significant IR bands and their assignments are reported in Tables 3.2. In Tables 3.3 and 3.4, TGA data of the compounds are presented.

3.2.6 Stability of the compounds in solution

Stability of the compounds in water was studied by estimating the peroxide content in aliquots drawn from a solution of the compounds (0.1 mM) at different time intervals by adopting methods described in Chapter **2**. As a measure of stability of the compounds in solution change in absorbances of their electronic spectral bands at 240-250 nm and molar conductances at ambient temperature were recorded at 30 min gap for a period of 12 hour. Stability of the compounds at pH 7.0 or 8.0 was measured similarly in compound solution (0.1 mM) in presence of phosphate buffer (50 mM, pH 7.0 or 8.0). In order to measure the stability of the compounds **3.5** and **3.6** at acidic pH citrate buffer was used (pH 3.4).

3.3 RESULTS AND INTERPRETATION

3.3.1 Synthesis and characterization

The methodology for the successful synthesis of the white microcrystalline dimeric compounds, **3.1** and **3.2** was based on the reaction of A_2WO_4 with 30% H_2O_2 and

$CysH_2$ in an aqueous acidic medium. Adopting a somewhat similar synthetic protocol the oxo-bridged pW compounds containing dipeptides as heteroligands, **3.3** and **3.4** were obtained from the reaction of H_2WO_4 with 30% H_2O_2 and the respective dipeptide ligand. Since one of our primary aims was to isolate complexes with a $[WO(O_2)_2]_2O$ moiety which has been reported to exist in an acidic solution[13], the pH of the reaction medium was strategically maintained at < 3. The factors such as maintenance of required time and temperature at ≤ 4 ^{0}C were found to be equally important for achieving the desired syntheses. Our attempts to isolate analogous dinuclear compounds in the presence of some other ligand systems such as proline, glutamic acid, asparatic acid, thioglycolic acid etc. were unsuccessful. For the formation of the monomeric molecular complexes **3.5** and **3.6**, a comparatively higher pH of *ca.* 6 of the reaction medium is likely to be responsible as it is known that in a solution of tungstate and excess H_2O_2 at a pH ≥ 5 diperoxotungstate species is readily formed.

In the solid state these complexes were found to be stable for several weeks stored dry at < 25 ^{0}C but tended to be hygroscopic at ambient conditions. The title compounds were diamagnetic in nature as was evident from the magnetic susceptibility measurement in conformity with the presence of tungsten (VI) in each case.

Valuable information regarding composition of the compounds was obtained from the elemental analysis data (Table 3.1). A ratio of 1:2 was obtained for W: peroxide in each of the compounds **3.1**-**3.6**. While W: cystine ratio was ascertained to be 2:1 in the compounds **3.1** and **3.2**, suggesting a dimeric nature of the complex species, the

Table 3.1 *Analytical data of the peroxotungstate compounds* **3.1-3.6**

Sl. No.	Compound	(% calculated) % found						Approximate Yield %	Molar Conductance
		Na/K	C	N	H	W	O_2^{2-}		($\Omega^{-1}cm^2\ mol^{-1}$)
1.	$Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ **(3.1)**	(4.91)	(7.69)	(2.99)	(1.28)	(39.31)	(13.67)		
		4.88	7.62	2.90	1.34	39.26	13.63	52	263
2.	$K_2[W_2O_3(O_2)_4(cystine)].4H_2O$ **(3.2)**	(8.05)	(7.43)	(2.89)	(1.24)	(38.01)	(13.22)		
		7.90	7.36	2.82	1.32	38.20	13.19	54	255
3.	$Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$ **(3.3)**	(5.06)	(10.57)	(6.16)	(2.20)	(40.52)	(14.09)		
		4.98	10.35	5.90	2.23	40.13	13.86	38	261
4.	$Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$ **(3.4)**	(4.50)	(18.82)	(5.49)	(3.52)	(36.07)	(12.54)		
		4.42	18.76	5.40	3.20	35.88	12.32	37	257
5.	$[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$ **(3.5)**	-	(10.27)	(5.99)	(3.42)	(39.40)	(13.70)		
		-	10.57	5.73	3.12	38.85	13.56	45	-
6.	$[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$ **(3.6)**	-	(18.32)	(5.34)	(4.58)	(35.11)	(12.21)		
		-	18.25	5.40	3.86	35.28	12.12	48	-

elemental analysis data indicated the presence of one dipeptide ligand per tungsten centre for the complexes **3.3-3.6**. The elemental analysis results and molar conductance values (Table 3.1) obtained from measurement at ambient temperature were in complete agreement with the formulation of the complex species as $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$ [A = Na(**3.1**) or K(**3.2**)], $Na_2[W_2O_3(O_2)_4(dipeptide)_2].3H_2O$ [dipeptide = glycyl-glycine (**3.3**) and glycyl-leucine, (**3.4**)] and $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$ [dipeptide = glycyl-glycine (**3.5**) and glycyl-leucine (**3.6**)], respectively.

The IR spectra of the title compounds (*Fig. 3.1 ,3.3 &3.5*) displayed a sufficiently well resolved spectral pattern main features of which are summarized in Tables 3.2. The strong absorption at *ca.* 960 cm^{-1} was consistent with the presence of a terminally bonded W=O group in each case[10,11]. For the dinuclear W-O-W unit the antisymmetric and symmetric stretching were expected in the 750-770 and 500 cm^{-1} regions[10,11], respectively. In the spectra of the dimeric complexes **3.1-3.4** a prominent band at *ca.* 770 cm^{-1} and another weak intensity band appearing at *ca.* 410 cm^{-1} attributable to ν_{asym} (W_2O) and and ν_{sym} (W_2O) modes gave clear indication of the presence of a bridging oxo group in each of them. The presence of side-on bound peroxo ligand in the compounds, was evident from the observance of the expected ν(O-O), ν_{as} and ν_s modes which involve metal-oxygen stretches of $W\text{-}O_2$ vibrations in the range of *ca.* 850, *ca.* 610 and *ca.* 530 cm^{-1}, respectively.

Table 3.2 *The structurally significant IR bands of the peroxotungstate compounds* ***3.1-3.6***

No.	Compound	IR bands							UV peaks	
		$\nu(W{=}O)$	$\nu(O\text{-}O)$	$\nu(W_2O)$	$\nu_{as}(W\text{-}O_2)$	$\nu_s(W\text{-}O_2)$	$\nu_{sym}(W_2O)$	$\rho_r(H_2O)$	nm	A(0.1 mM)
3.1	$Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$	962	878	744	605	520	410	-	246	0.60
3.2	$K_2[W_2O_3(O_2)_4(cystine)].4H_2O$	957	875	744	605	518	415	-	244	0.59
3.3	$Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$	952	843	770	612	531	410	-	242	0.58
3.4	$Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$	949	845	767	613	534	415	-	243	0.56
3.5	$[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$	957	875	-	615	539	-	725	244	0.54
3.6	$[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$	956	876	-	593	540	-	728	247	0.57

IR spectroscopy is immensely useful and informative for the characterization of heteroligand peroxometal complexes since it provides information not only about co-ordination of peroxo but also ligand groups when compared with the spectra of the free ligands. The characteristic shifts of heteroligand bands that occur upon co-ordination compared to the spectra of the free ligand reveal the bonding sites in the co-ordinated ligands.

The existence of coordinated cystine in the complexes **3.1** and **3.2** was evident from their IR spectra, which showed characteristic differences between the spectral pattern originating from the complexes and those of free cystine and cysteine[15]. The absorption attributable to ν_{as}(COO) of coordinated carboxylate group appeared at *ca.* 1640 cm^{-1} in the spectra of the complexes. The symmetric stretching vibration of the carboxylate group of unco-ordinated ligand occurs at 1430 cm^{-1}. The spectra of the compounds displayed a medium intensity band at ca. 1392 cm^{-1} which may be assigned to ν_s(COO) . The shifting of this band to lower frequency with the difference of ν_{as} - $\nu_s \approx$ 250 cm^{-1} is characteristic of monodentate coordination of the carboxylate group via the O(carboxylate) atom[16]. Significant is the absence of S-H stretching (at *ca.* 2565 cm^{-1}) in the spectra of the complexes, thereby implying either oxidation of the sulfhydryl group to its disulfide form or its deprotonation and subsequent co-ordination[15]. However, since no band attributable to ν(W-S) has been observed at *ca.*390 cm^{-1} region[15], participation of S atom in co-ordination has been ruled out. On the other hand, the spectra of the complexes exhibited an intense band at 538 cm^{-1} characteristic of –S-S- moiety[15], thereby causing us to infer that the amino acid is present in the complex in its disulfide form i.e. as cystine and not cysteine. The bands appearing in the 3200-3000 cm^{-1} region and at *ca.*1500 cm^{-1}

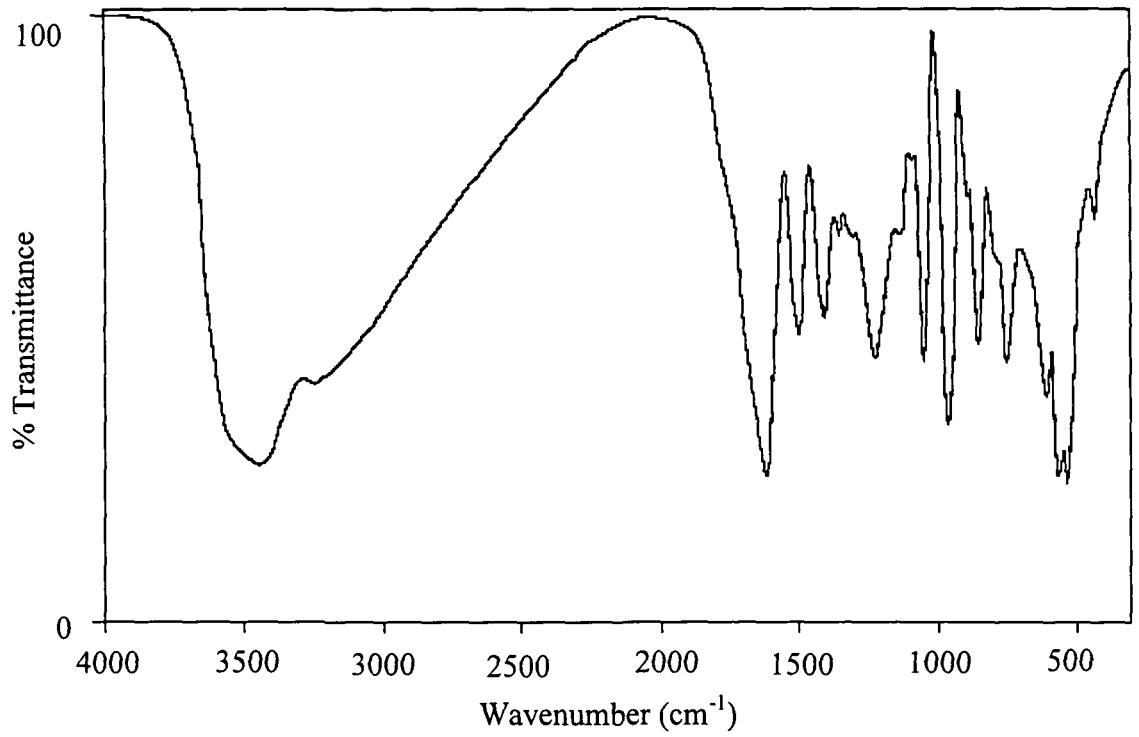


Fig. 3.1 IR spectrum of $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$(**3.1**)

were assigned to the N-H stretching and N^+H_3 symmetric deformation modes of the N^+H_3 group, respectively. The bands appearing in the 3200-3000 cm^{-1} region and at *ca.*1500 cm^{-1} were assigned to the N-H stretching and N^+H_3 symmetric deformation modes of the N^+H_3 group, respectively. The rocking modes of N^+H_3 occurred at *ca.*1190 and 1049 cm^{-1}. It was thus evident that N atom do not participate in co-ordination in the title compounds. Presence of lattice water in the complexes **3.1** and **3.2** was indicated by the strong ν(OH) absorptions displayed at 3500-3400 cm^{-1}. However, the bending mode of water could not be assigned with certainty as it occurred in the carbonyl frequency

region. The IR spectral data thus suggest that in the complexes **3.1** and **3.2**, cystine occurring as zwitterion binds the W(VI) centres through O(carboxylate) atoms.

The above results are consistent with a structure of the complex species shown in *Fig. 3.2*. In the dinuclear oxobridged complex ion the ancillary ligand acts as a bridge between the two peroxotungstate moieties thereby completing hepta coordination around each of the tungsten (VI) centres.

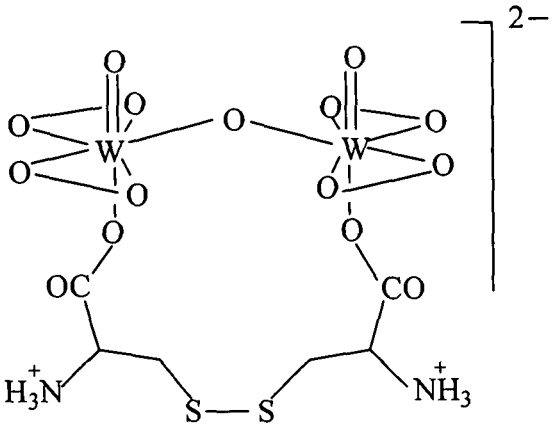


Fig. 3.2 A proposed structure of complex **3.1**, $[W_2O_3(O_2)_4(cystine)]^{2-}$

The IR spectra of glycyl-glycine and its compounds have been extensively studied in solution[17-20] as well as in solid-state[17]. The spectra of the peptide containing dinuclear pW complexes **3.3** and **3.4** as well as monomeric compounds **3.5** and **3.6** exhibited two distinct bands in the range of 1680-1660 cm^{-1} and 1630-1590 cm^{-1} which have been assigned to ν(C=O) (amide) and ν_{as}(COO) of the co-ordinated peptide ligands[17,18,18]. The

position of ν(C=O) band in the complexes almost remained unaltered compared to its position in free ligand, which indicated that the amide group was not taking part in co-ordination. The broadening of the band was probably owing to their participation in hydrogen bonding. Co-ordination through N-atom of the amide group was unlikely as evident from the spectra because such co-ordination is known to cause considerable decrease in the peptide carbonyl stretching frequency, which was not observed in case of these compounds[21,22]. The ν_s(COO) vibration of the free ligands were observed in the range of 1410–1400 cm^{-1} in the IR spectra[17]. A medium intensity band with some broadening observed in the range of 1395-1405 cm^{-1} region was assigned to ν_s(COO) of the unidentate carboxylate group (ν_{as}–$\nu_s \approx 300\ cm^{-1}$)[17]. The broadening of the band was probably caused by its mixing with the C–N stretching of amide group expected to occur in this region[19]. The spectra showed N-H stretching bands of coordinated peptide residue at 3300-3100 cm^{-1} region as expected from the $-N^+H_3$ group. N–H deformation modes (1520-1600 cm^{-1}) in their position and pattern in the spectra of the complexes significantly remained unaltered compared to the free ligand. Presence of lattice water in each of the complexes **3.3** and **3.6** was indicated by the strong ν(OH) absorptions displayed at 3500-3400 cm^{-1}. However, the bending mode of water could not

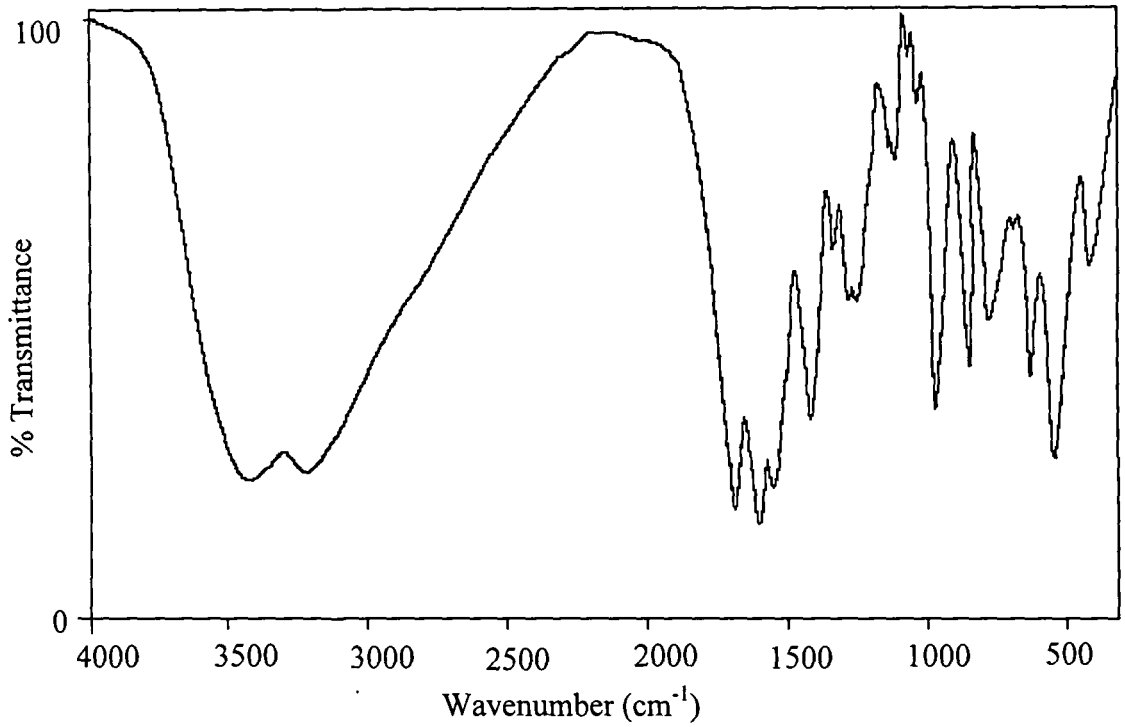


Fig. 3.3 IR spectrum of $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$ (**3.3**)

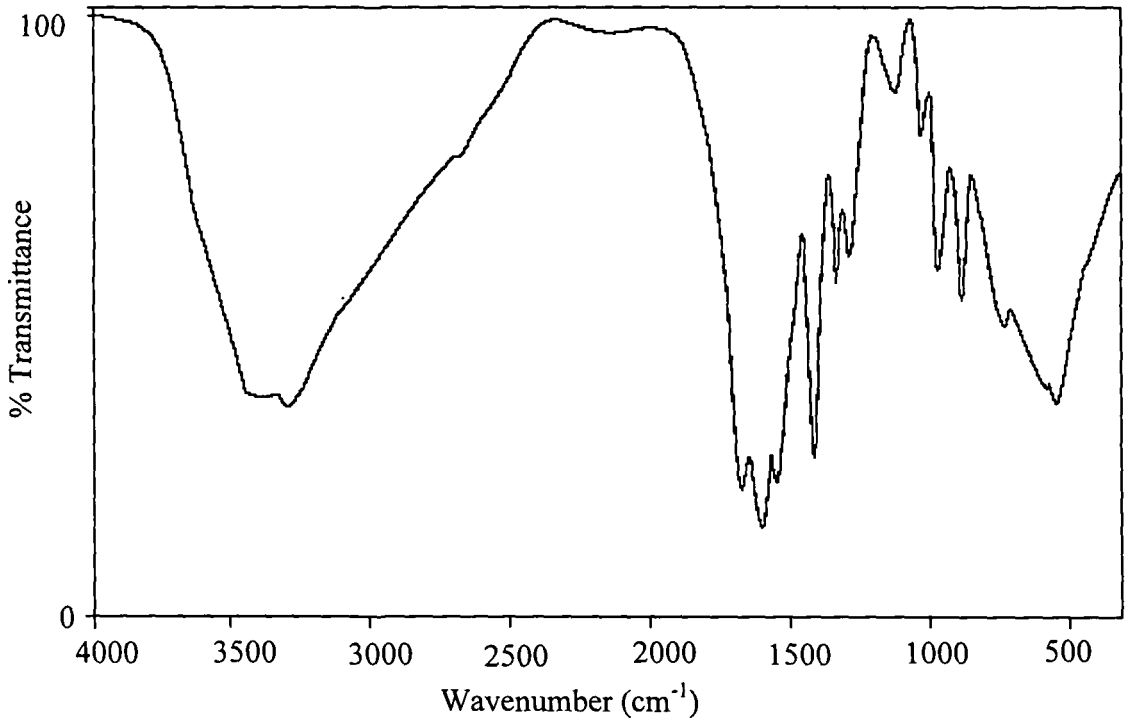


Fig. 3.4 IR spectrum of $[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$ (**3.5**)

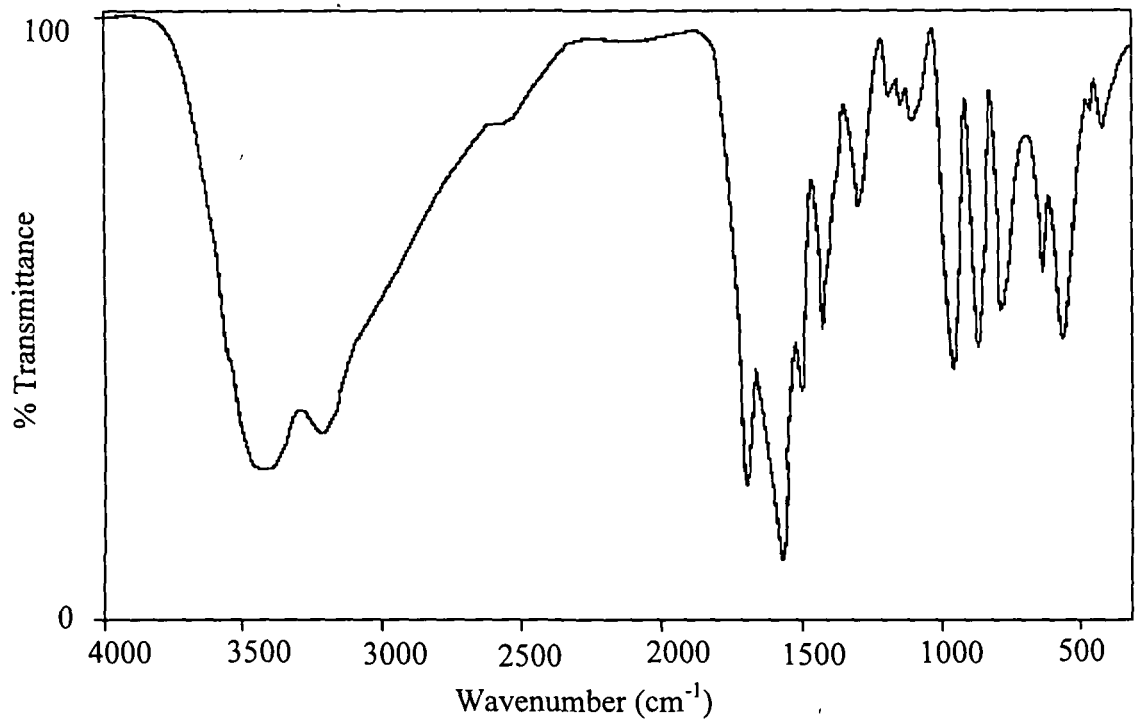


Fig. 3.5 IR spectrum of $Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$ (**3.4**)

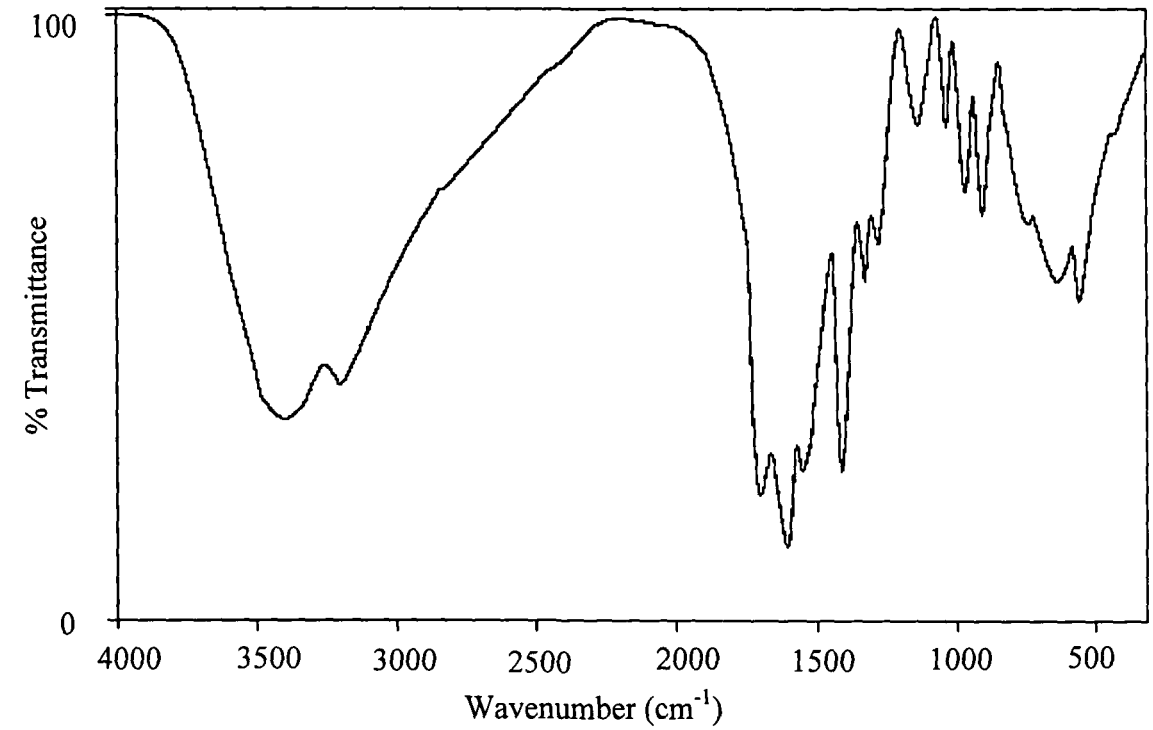


Fig. 3.6 IR spectrum of $[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$ (**3.6**)

be assigned with certainty as it occurred in the carbonyl frequency region. In the spectra of the compounds **3.5** and **3.6**, a consistent appearance of a medium intensity signal at *ca.* 725 cm^{-1} attributable to rocking mode of water indicated the presence of co-ordinated water in these compounds. The IR spectral data thus suggest that in each of the complexes **3.3** to **3.6**, dipeptide ligand occurring as zwitterion binds the metal center through O(carboxylate) atom. A co-ordinated water molecule completes hepta co-ordination in the monomeric pW compounds **3.5** and **3.6**.

Electronic spectra of compounds **3.1** to **3.6** in aqueous solution (*Fig. 3.6-3.8)* exhibited a weak intensity broad band at 240-250 nm (Table 3.2) originating from co-ordinated peroxide. These bands are typical of LMCT transitions of diperoxo derivatives of tungsten[1,23].

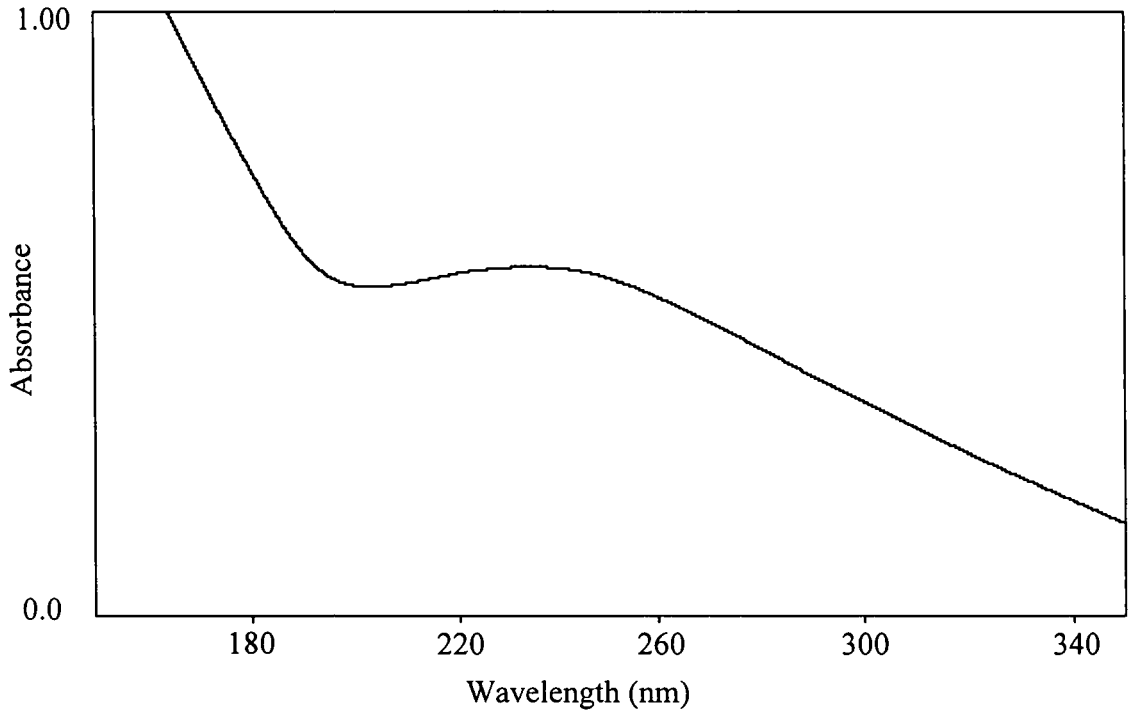


Fig. 3.6 UV Spectrum of $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ (**3.1**), (0.1mM)

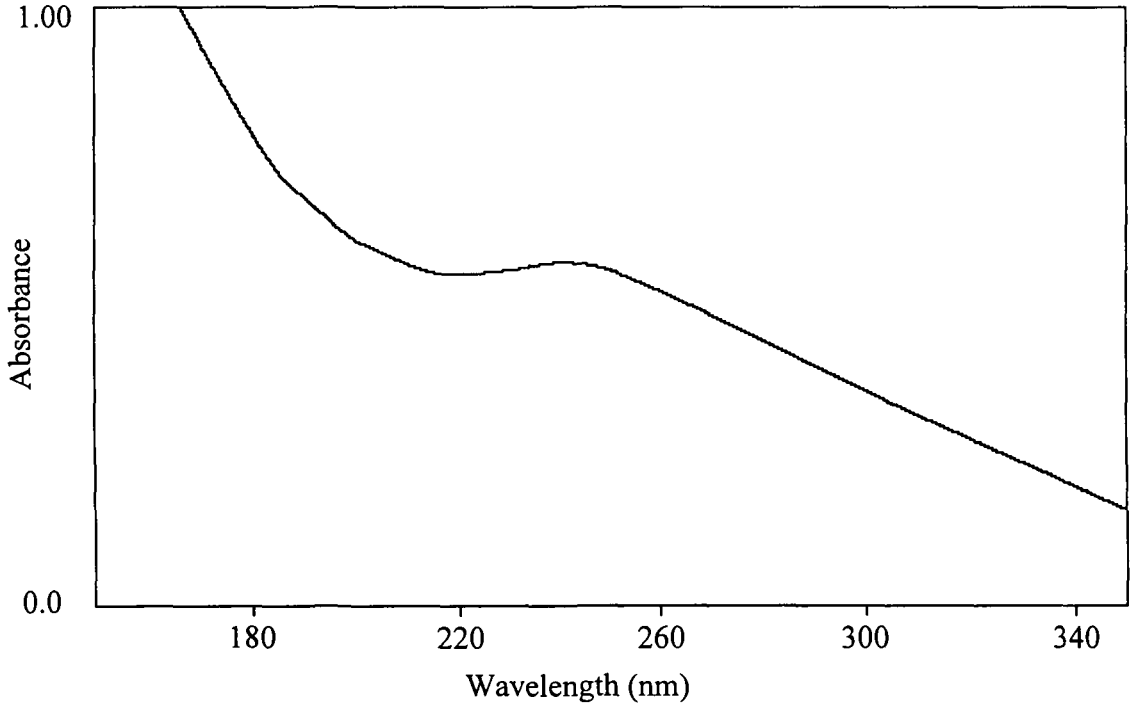


Fig. 3.8 UV Spectrum of $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$ (**3.3**)

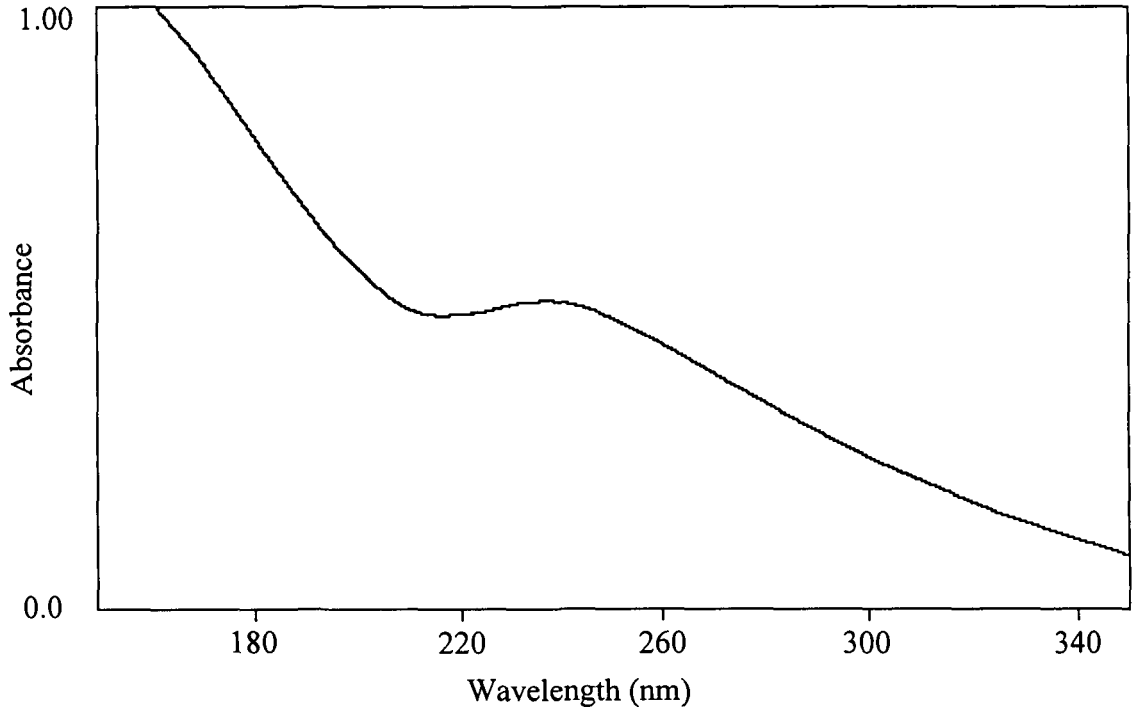


Fig. 3.9 UV spectrum of $[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$ (**3.5**), (0.1mM)

Based on these data proposed structure applicable to the complexes **3.3** and **3.4** is shown schematically in *Fig. 3.10*. For complexes **3.5** and **3.6** the structure of the type shown in *Fig. 3.11* has been envisaged.

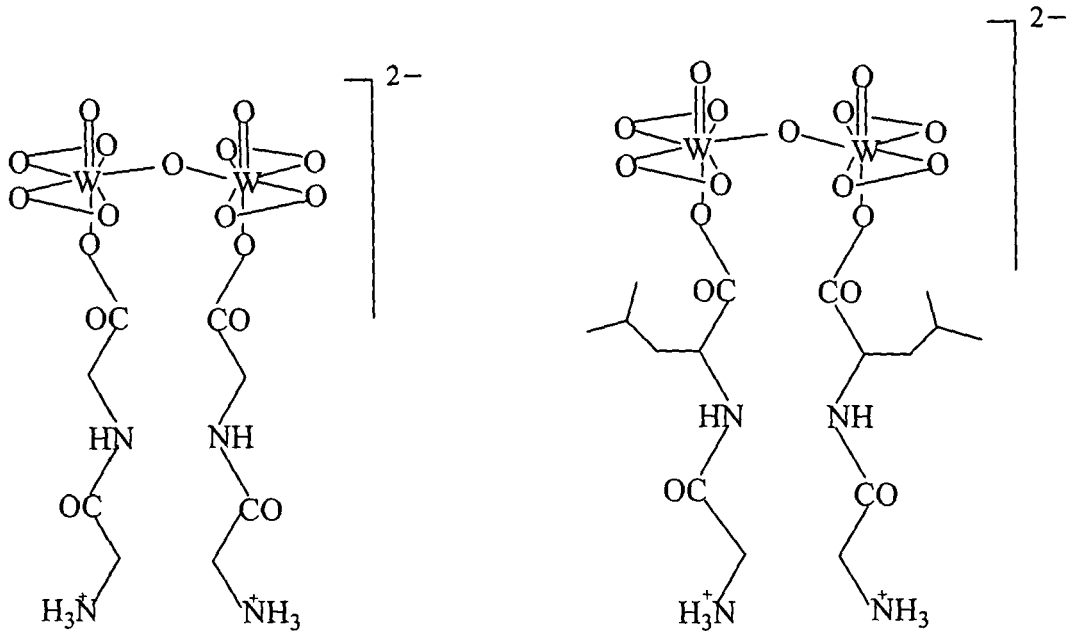
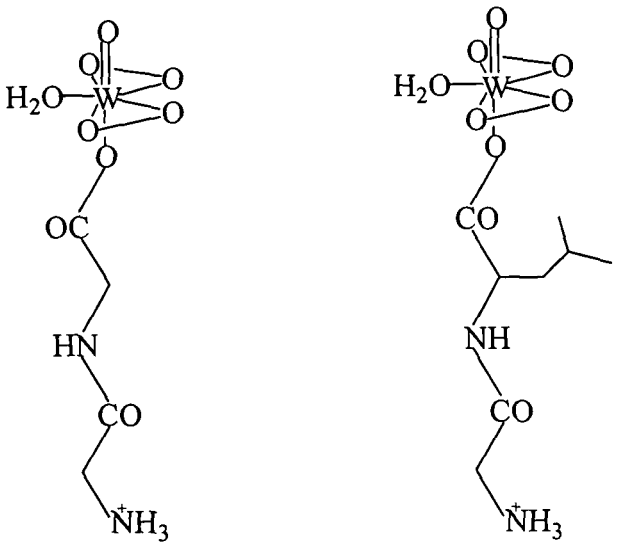


Fig. 3.10 A representation of proposed structure of the dinuclear peroxotungstate complex anions (a) $[W_2O_3(O_2)_4(gly\text{-}gly)_2]^{2-}$; (b) $[W_2O_3(O_2)_4(gly\text{-}leu)_2]^{2-}$



(a) (b)

Fig. 3.11 A representation of proposed structure of the mononuclear peroxotungstate complexes (a) $[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$; (b) $[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$

3.3.2 Thermal analysis

Thermo-gravimetric analysis data indicated that after the initial dehydration, each of the title compounds undergoes multistage decomposition up to a final decomposition temperature of 550 ^{0}C. The complexes do not explode on heating.

The TGA curve of the dimeric cystine-containing compound **3.1** shows (*Fig. 3.12*) the first stage of decomposition occurring between the temperature range of 40-100 ^{0}C (Table 3.3) with the liberation of the outer sphere water molecules from the complex. The corresponding observed weight loss of 6.7 % is in good agreement with the calculated values of 7.6 %. The compound subsequently undergoes decomposition with the formation of unstable decomposition products. The total weight loss which occurred during the course of the overall decomposition process on heating the compounds up to a

Table 3.3 *Thermal decomposition of dinuclear peroxotungstate (VI) complexes* **3.1 - 3.4**

Sl. No	Compound	Temperature range (^{0}C)	% Weight loss					
			H_2O		O_2^{2-}		Total loss	
			Found	Calcd	Found	Calcd	Found	Calcd
1.	$Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ **(3.1)**	50-105	6.7	7.6	-	-		
		110-260	-	-	14.5	13.6		
		up to 550					44.9	45.9
2.	$K_2[W_2O_3(O_2)_4(cystine)].4H_2O$ **(3.2)**	40-100	6.7	7.4	-	-		
		120-240	-	-	12.8	13.2		
		up to 550					43.7	44.6
3.	$Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$ **(3.3)**	50-100	5.4	5.9	-	-		
		110-200	-	-	13.2	14.1		
		up to 550					48.1	49.1
4.	$Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$ **(3.4)**	50-90	5.0	5.2	-	-		
		100-160	-	-	11.5	12.5		
		up to 550					54.1	53.7

Table 3.4 *Thermal decomposition of mononuclear peroxotungstate (VI) complexes* **3.5** *and* **3.6**

Sl. No	Compound	Temperature range (^{0}C)	% Weight loss					
			H_2O(outer sphere)		O_2^{2-} + H_2O(coordinated)		Total loss	
			Found	Calcd	Found	Calcd	Found	Calcd
1.	$[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$ **(3.5)**	30-110	10.3	11.5	-	-		
		110-240	-	-	17.4	17.5		
		up to 550					61.0	59.4
2.	$[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$ **(3.6)**	30-100	9.2	10.3	-	-		
		100-220	-	-	14.5	15.6		
		up to 550					58.7	60.9

final temperature of 600 ^{0}C was recorded to be 44.9 %, in good agreement with the theoretically calculated value of 45.96 % for the complete loss of the components viz. water molecule, co-ordinated peroxide, and the cystine ligand. The residue remaining after heating the compound up to 550 ^{0}C were found to be an oxo-tungsten species.

The initial decomposition for the peptide containing monomeric and dimeric pW complexes **3.3** - **3.6** occurs between room temperature and a temperature of 100 ^{0}C with the release of the three crystallized water molecules from the complexes (Table 3.3 and 3.4). Another decomposition stage observed in the temperature region of 110-200 ^{0}C (*Fig. 3.13*) for complex **3.3** and 90-160 ^{0}C (*Fig. 3.14*) for complex **3.4** with a corresponding weight loss of 13.2% and 11.5% respectively is characteristic of complete loss of co-ordinated peroxo groups from the complexes[12]. Significantly, in case of compounds **3.5** and **3.6** the initial loss of lattice water is followed by a two step decompo-

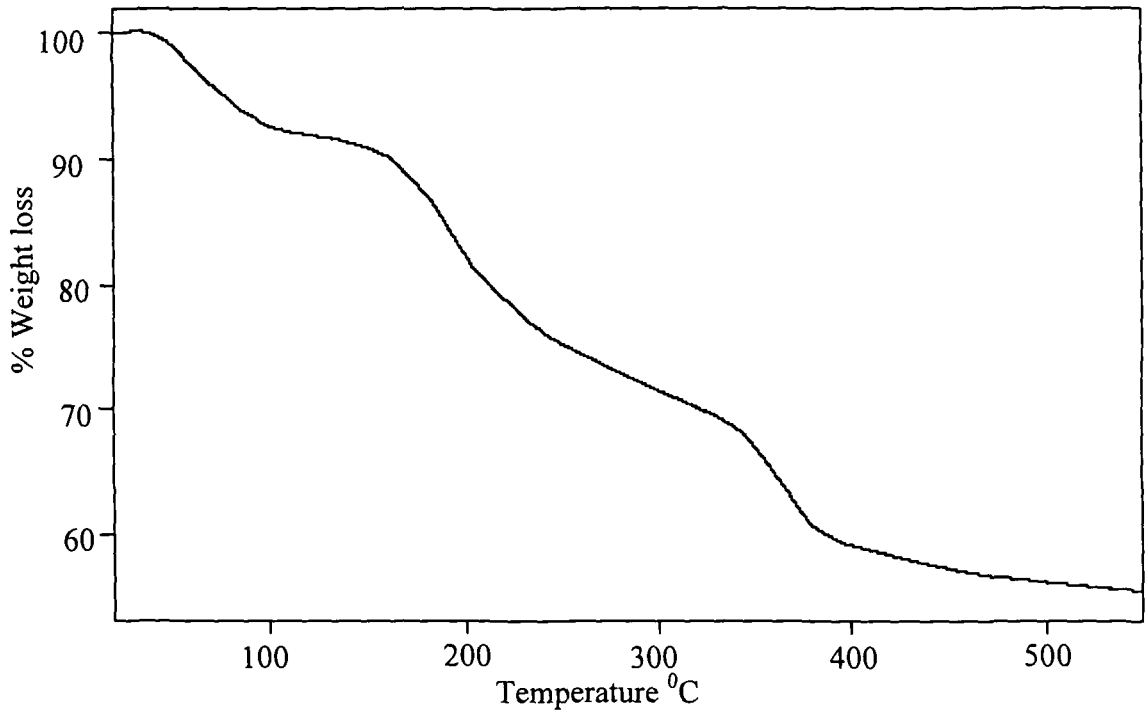


Fig. 3.12 TGA curve of $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ (**3.1**)

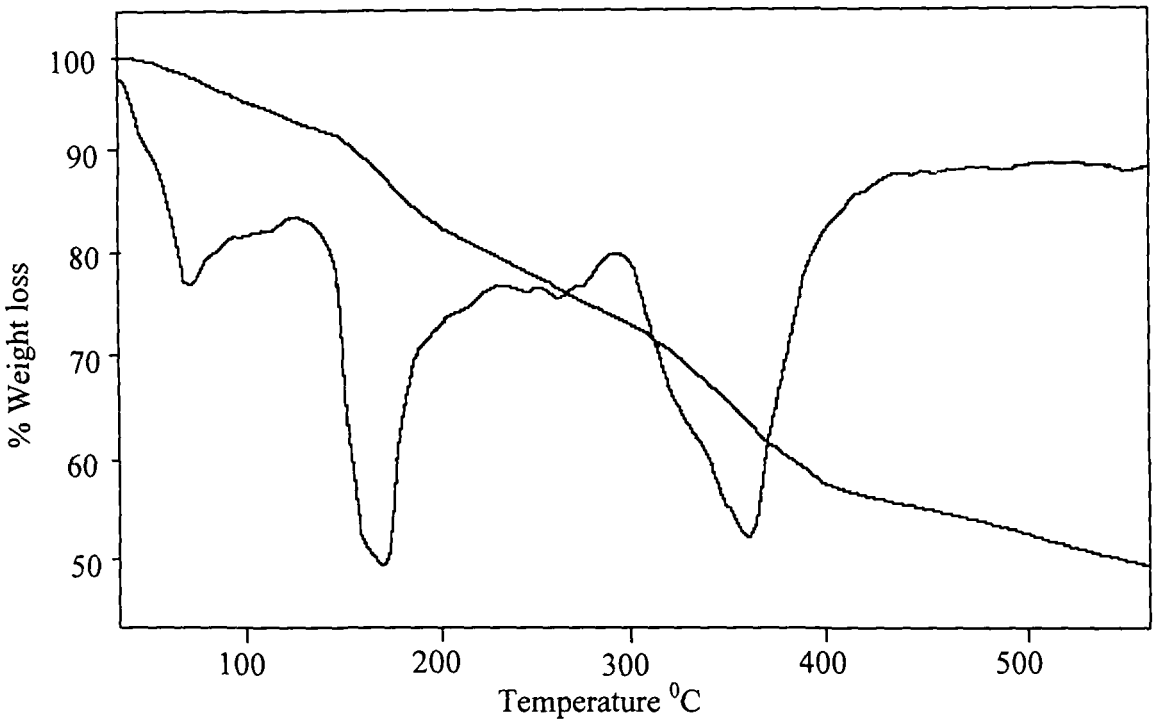


Fig. 3.13 TGA/DTG curve of $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$(**3.3**)

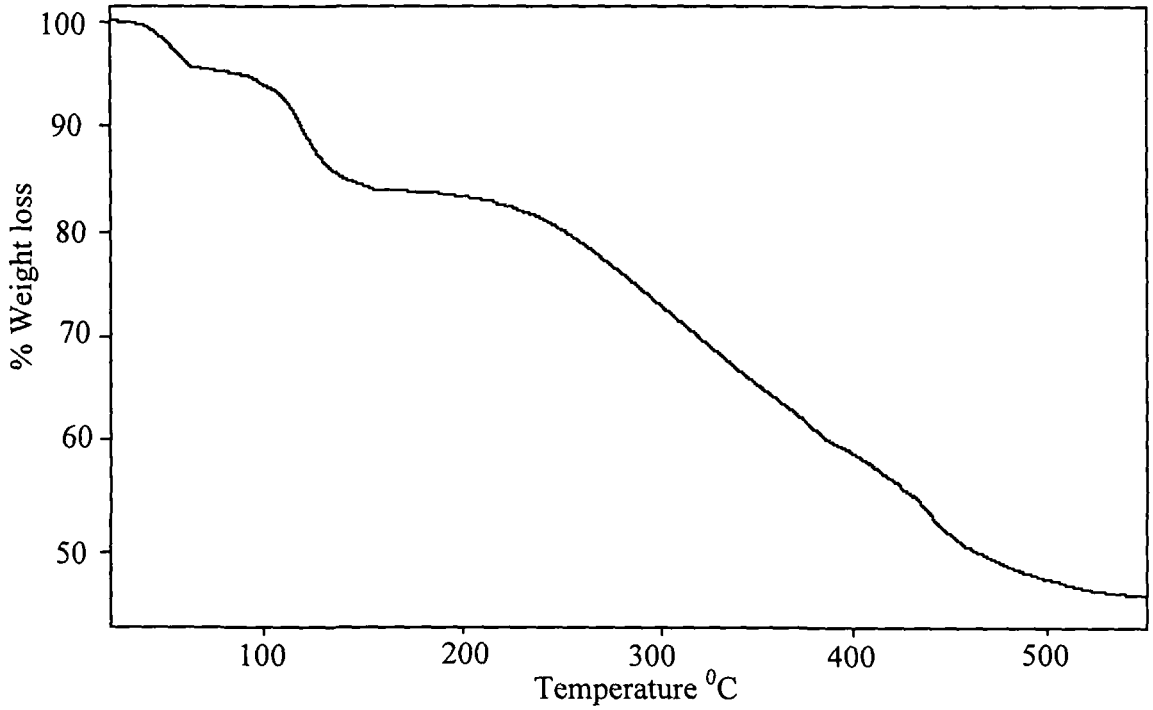


Fig. 3.14 TGA curve of $Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$ (**3.4**)

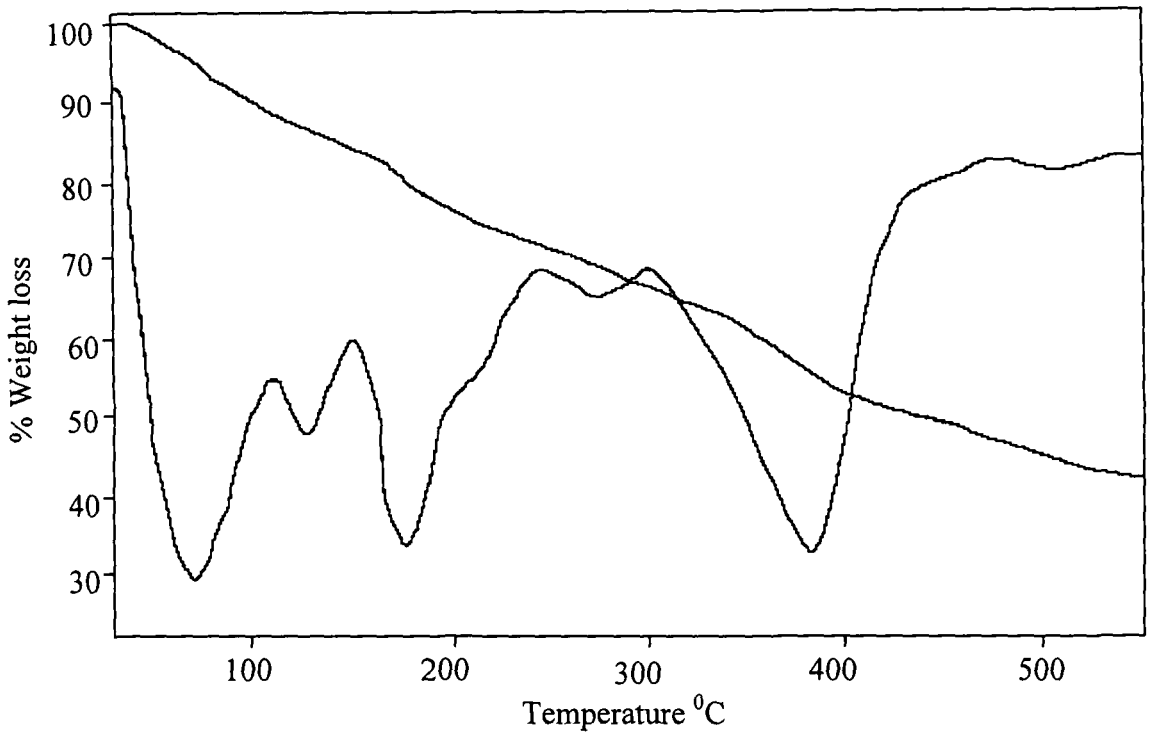


Fig. 3.15 TGA/DTG curve of $[WO(O_2)_2(glyl\text{-}gly)(H_2O)].3H_2O$(**3.5**)

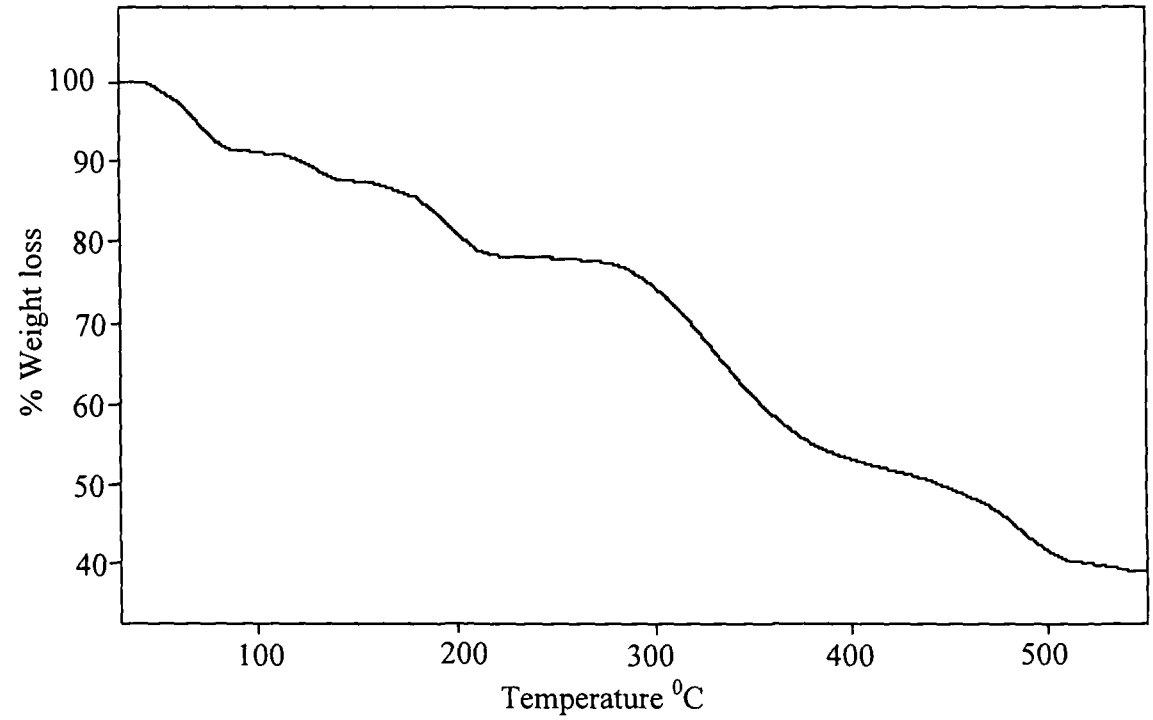


Fig. 3.16 TG curve of $[WO(O_2)_2(glyl\text{-}leu)(H_2O)].3H_2O$(**3.6**)

sition stage in the temperature range of 110-240 ^{0}C (*Fig. 3.15* and *Fig. 3.16*) attributable to loss of co-ordinated water and peroxo groups from the complexes. The corresponding observed weight loss agreed very well with the calculated values for the loss of one molecule of coordinated water and peroxide groups, consistent with the formula assigned for the complexes. The results thus evidenced for the presence of co-ordinated, as well as lattice water in the compounds. Absence of peroxo group in the decomposition product was confirmed from the infrared spectral analysis. Mention must be made that some peroxotungstate and peroxomolybdate compounds containing amino acids as co-ligands were reported to behave in a similar manner losing their peroxide completely on heating up to a temperature of 240-250 ^{0}C [12]. In the present study, the loss of peroxide is seen to be followed by another decomposition stage occurring between 240-550 ^{0}C. TGA data obtained for this decomposition indicated the loss of coordinated dipeptide ligands from the complexes.

The total weight loss recorded during the course of the overall decomposition process for each of the compounds **3.3-3.6** was observed to be consistent with the theoretically calculated value for the complete loss of the components viz, lattice water, co-ordinated peroxide, and the dipeptide ligands. The dark brown sticky residue remaining in each case after heating the compounds up to 550^{0}C was found to be a hydrated oxotungstate species as indicated by the IR spectrum which displayed the characteristic ν(W=O) and ν(OH) absorptions and was devoid of bands attributable to peroxo and the dipeptide ligands of the original compounds. Thermogravimetric analysis data of the compound thus provided further evidence in support of their composition and formula assigned.

3.3.3 Stability of the complexes in solution

The stability of the compounds with respect to the loss of peroxide in solution at pH *ca.*3 (for compounds **3.1-3.4**) or *ca.*5 (compounds **3.5** and **3.6**), the natural pH attained by the solution on dissolving the compounds, has been studied by estimating their peroxide content and absorbances at 240-250 nm region in the electronic spectra at specified time intervals for any possible change. The investigations revealed that their peroxide content and position and intensity of their electronic spectral bands remained unaltered for over a period of 12 hours (shown in Chapter **5**, *Fig 5.2*). Furthermore, the molar conductances of anionic dimeric complexes **3.1** to **3.4** recorded at ambient temperature after 12 h of solution preparation showed no appreciable change. We further examined and ascertained their stability in solutions of pH values of 7.0 and 8.0 in phosphate buffer. Moreover, the neutral monomeric compound **3.5** and **3.6** were observed to be stable at pH 3.8 in citrate buffer.

3.4 DISCUSSION

The importance of pH for the successful synthesis of peroxo-metal compounds has been emphasized in the literature[1,10,11]. It has been known that a number of peroxotungsten species are formed in solution with slight variation of pH of the reaction medium[1,10]. In the present study, it has been possible to isolate two types of peroxotungstates viz., anionic oxo-bridged complex species $[W_2O_3(O_2)_4(L)_2]^{2-}$, (L = cystine, gly-gly or gly-leu) and molecular monomeric complexes $[WO(O_2)_2(L)($

$H_2O)].3H_2O$ (L = gly-gly or gly-leu), by conducting the reaction under two different, in each case specific, pH conditions. The essential parameters for achieving success of the synthesis of the oxo-bridged dinuclear peroxotungsten complexes were the use of the acidic medium and proper choice of the ancillary ligands. The compounds **3.1-3.4** are probably the first known examples of dimeric oxo-bridged compounds isolated into solid state having a co-ligand other than water. The fact that such compounds could so far be isolated only with cystine or the glycyl peptides as heteroligand suggests that the chosen amino acid or dipeptide ligands may have a role in stabilizing the products. It is reasonable to expect enhanced stability of the tungsten dimer due to the bridging cystine in complxes **3.1** and **3.2** and possibility of hydrogen bond interaction with the amide groups of the side chain in the dipeptide ligands.

Cysteine is a major metal binding site in proteins, which undergo oxidation to the disulfide form, a reaction that may be catalyzed by metal ions or other oxidant species present. Oxidation of cysteine to cystine during the course of the reaction in the present case is not really unexpected under the reaction conditions used especially in presence of abundant H_2O_2.

Depending on the pH of the reaction solution and the nature of the metal, an amino acid such as cystine or a dipeptide provides several alternative coordination sites to the metal, viz., terminal amino, carboxylate, as well as amide linkage in case of peptide, and the S atoms of cystine[15,15,24-26] Thus the chosen species are susceptible to act as mono, bi or tridentate ligands with different combinations of donor atoms and can occur in complexes in either neutral zwitterionic form or anionic form. In addition, they are known to form bridges between metal atoms leading to the formation of dinuclear or

polynuclear structures[15,24-26]. In the present study, the acidic pH apparently favored the co-ordination of cystine, in its neutral zwitterionic form to the two W(VI) centers through carboxylate groups thereby stabilizing the $[W_2O_3(O_2)_4]^{2-}$ moiety and leading to the synthesis of the desired complexes. Similarly, the relatively low pH of the reaction medium maintained in the range of 2.5 -5.5 appears to be responsible for occurrence of the peptides as zwitterions and their preferential co-rdination through carboxylate group to W(VI), in each of the monomeric and dinuclear pW compounds.

The high stability of the compounds in solution at acidic as well as physiological pH is likely to be a consequence of the additional stability imparted by the heteroligands. It seems likely that the nature of the heteroligand has a specific effect on the thermal stability of the complexes as reflected in the variation in their decomposition temperatures with respect to the loss of peroxide.

In summary, with the examples of the newly synthesized dinuclear complexes the present investigation has established that it is possible to isolate the oxo-bridged tetraperoxotungstate(VI) species formed in solution into solid state, through complexation with specific ligands under appropriate experimental conditions. A noteworthy finding of the present investigation, which may also be of biochemical importance, is the high stability of the compounds in solution at a wide range of pH particularly at acidic pH. Results of investigation on the interaction of the title compounds with the enzyme catalase and their effect on the alkaline phosphatase activity, as well as their redox properties are presented in Chapters **5** and **6** of the thesis.

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CHAPTER 4

Synthesis and characterization of new peroxovanadium compounds with dipeptides as heteroligands. Studies on their nature and stability in solution*

4.1 INTRODUCTION

The chemistry of peroxovanadium complexes, as mentioned in Chapter1, has been of prime interest owing mainly to their relevance to the activity of haloperoxidases[1-3], their enzyme inhibitory [3], antineoplastic and insulin like effects[4-21] and potent catalytic properties[3,22-33]. The mechanisms by which pV species generate the large number biological and biochemical responses are yet to be understood completely[34-36]. Co-ordination chemistry of vanadium plays a central role in the interaction with biomolecules and hence identification of the actual processes involved and the function of vanadium in complex biomolecules require an understanding of the basic chemistry of vanadium, particularly their interaction with simpler ligands.

Despite the variety and number of heteroligand pV complexes that has been synthesized in recent years[6,22,34,37,38] and the intense biological work and solution studies carried out on interaction of vanadates with biogenic species viz., amino acids, peptides and proteins[39-42], information pertaining to well characterized synthetic pV complexes with co-ordinated peptides are very few[43-47]. Peptides are probably the primary ligands to interact with vanadyl and vanadate in biological systems. A better insight into the compl-

* Results described in this Chapter have been accepted for publication in:
Trans. Met. Chem., 2007 (in press).

exation behaviour of vanadium with such ligands is therefore of vital interest. Vanadium binds specifically and non-specifically to various proteins including carboxy peptidase, nuclease and phophatases[48,49].

Pertinent here is to mention that for more than half a decade our group has engaged its attention to devise synthetic strategies and to make systematic studies of peroxo-metal compounds with physiologically relevant molecules acting as ancillary ligands[43-45,47]. A series of peroxo-bridged divanadate compounds of the type $[V_2O_2(O_2)_3.(L)_3].H_2O$ (L = amino acid or dipeptide) synthesized previously in our laboratory, exhibited unique redox properties and could efficiently mediate oxidative bromination of organic substrates at physiological pH[43,44]. Diperoxovanadate compounds with exclusively η^2-peroxo groups in its co-ordination sphere were found to be catalytically incompetent in bromide oxidation at neutral pH. The μ-peroxo V compounds however, undergo rapid degradation in solution with loss of its high bromination activity. It is notable that as already mentioned in the introductory Chapter, most of the pV compounds tested for therapeutic potential are hydrolytically unstable which limits their utility[36,50].

Thus the current intensive search for bio-relevant pV compounds possessing appropriate properties of solubility and stability towards decomposition in solution and the paucity of information on peptide containing peroxo-vanadium compounds, motivated us to direct our efforts to synthesize newer pV compounds stabilized by dipeptides functioning as ancillary ligands. Since we have already gained an access to dimeric and monomeric diperoxotungsten compounds with gly-gly and gly-leu as ancillary ligands (compounds **3.1-3.6**), we were particularly interested to obtain

diperoxovanadate(V) compounds in analogous co-ligand environment as such systems would enable us to compare the complexes of the two metals viz., V and W in terms of their stability and other biochemically relevant properties. There appears to be no literature comparing these systems.

In this Chapter, the synthesis and physicochemical characterization of new diperoxovanadate complexes, $A[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$, A= Na (**4.1**) or K (**4.2**) and $A[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$, A=Na (**4.3**) or K (**4.4**) are reported. Results of investigation on the nature and stability of the newly synthesized compounds in solution are also reported herein.

4.2 EXPERIMENTAL SECTION

4.2.1 Synthesis of monomeric peroxovanadate complexes, $A[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$, A= Na (4.1) or K (4.2) and $A[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$, A=Na (4.3) or K (4.4).

In a typical procedure representative of the general method, solid V_2O_5 (0.25 g, 1.37 mmol) was mixed with the respective dipeptide (0.180 g, 1.37 mmol) in a 250 ml beaker. To this mixture 12 ml of 30% H_2O_2 (105.84 mmol) was added gradually with constant stirring. A clear reddish yellow solution of pH *ca.*2 was obtained after stirring the mixture in an ice bath for *ca.*15 min. The corresponding alkali metal hydroxide solution (0.1 M) was then added drop wise with constant stirring to raise the pH of the solution to *ca.*5. Addition of pre-cooled ethanol (about 50 ml) to this mixture under vigorous stirring afforded a yellow colored pasty mass. After decantation of the

supernatant liquid, the residue was treated repeatedly with acetone in a manner analogous to that mentioned under the synthesis of pW compounds in Chapter **3**. The yellow microcrystalline product obtained was separated by centrifugation, washed with cold ethanol and finally dried under vacuum. The compounds are hygroscopic however, remain stable for several weeks when stored dry at <20°C.

4.2.2 Elemental analysis

The compounds were analyzed for vanadium, peroxide, carbon, hydrogen and nitrogen by the methods mentioned in Chapter 2. The analytical data of the compounds are summarized in Table 4.1.

4.2.3 Physical and spectroscopic measurements

Spectroscopic measurements, molar conductances and thermogravimetric analysis were performed by using instruments and methods described in Chapter **2**. Structurally significant IR and UV bands and their assignments are reported in Table 4.2 and 4.3. Thermogravimetric analysis data are presented in Table 4.4.

4.2.4 Nature and stability of the compounds in solution

Stability of the compounds in solution was tested by procedures outlined in Chapters **3**. In addition, ^{51}V-NMR spectroscopy has been used for ascertaining the nature of the compounds in solution.

4.3 RESULTS AND INTERPRETATION

4.3.1 Synthesis and characterization

Given to undergo favourable condensation reactions with H_2O_2 in aqueous solutions, vanadate yields peroxovanadates with increasing ratio of peroxide/V on increasing pH and reagent concentration[51,52,53]. In the present study, the yellow microcrystalline peroxo-vanadate compounds **4.1-4.4** were isolated from the reaction of V_2O_5 with H_2O_2 and the respective dipeptide by raising the pH value to *ca.*5. The alkali used to adjust the pH of the solution served also as a source of counter cation Na^+ or K^+.

The pV compounds reported herein are diamagnetic in nature in conformity with the oxidation state of the metal. The elemental analysis data for the compounds **4.1-4.4** indicated the presence of two peroxide groups and one peptide ligand per metal centre. The results of conductance measurements (Table 4.1) and elemental analysis were compatible with the formulation of the complex species as anionic diperoxovanadates, $A[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$, A= Na (**4.1**) or K(**4.2**) and $A[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$, A =Na (**4.3**) or K (**4.4**), respectively.

The compounds exhibited characteristic spectral pattern in the IR region (*Fig. 4.1& 4.2*) showing the presence of co-ordinated oxo, peroxo and co-ordinated peptide in each of them. The significant general features are presented in Table 4.2. The occurrence of side-on bound peroxo ligand in these compounds was evident from the observance of

Table 4.1 *Analytical data of the peroxovanadate compounds* **4.1-4.4**

Sl. No.	Compound	(% calculated) % found						Approximate Yield %	Molar Conductance
		Na/K	C	N	H	V	O_2^{2-}		($\Omega^{-1}cm^2\ mol^{-1}$)
1.	$Na[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$ **(4.1)**	(7.16)	(14.95)	(8.72)	(3.42)	(15.88)	(19.93)		
		7.14	14.76	8.65	3.23	15.56	19.50	43	132
2.	$K[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$ **(4.2)**	(11.57)	(14.24)	(8.30)	(3.26)	(15.13)	(18.99)		
		11.32	14.22	8.23	3.12	15.14	18.54	40	128
3.	$Na[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$ **(4.3)**	(6.10)	(25.46)	(7.42)	(5.03)	(13.52)	(16.97)		
		6.02	25.44	7.35	4.23	13.25	16.58	43	138
4.	$K[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$ **(4.4)**	(9.92)	(24.42)	(7.12)	(5.34)	(12.97)	(16.28)		
		9.32	24.40	7.09	4.49	12.74	16.14	40	135

the typical ν(O-O), ν_{as} and ν_s modes, which involve metal-oxygen stretches of V-O_2 vibrations[54] in the vicinity of *ca.* 870, *ca.* 610 and *ca.* 530 cm^{-1}, respectively. The spectra enabled clear identification of ν(M=O) near 960 cm^{-1} arising from terminally bonded V=O group[52,52].

In addition to the features originating from VO^{3+} and co-ordinated peroxide, the pV compounds displayed bands due to the co-ordinated dipeptide ligand, gly-gly or gly-leu. The spectral patterns originating from co-ordinated peptides of the pV compounds **4.1** to **4.4,** compared very well with those observed for corresponding pW compounds **3.4-3.6** reported in Chapter **3**.

In the spectra of the complexes **4.1- 4.4** a band occurring in the 1680-1670 cm^{-1} region has been assigned to ν(C=O)(amide) of co-ordinated peptide ligand. Since there was no notable change in its position compared to free ligand value, it may be inferred that amide group is not involved in co-ordination[55,56]. The absorption attributable to ν_{as}(COO) was observed between 1630-1600 cm^{-1}. The symmetric stretching vibration of the carboxylate group of uncoordinated glycyl-glycine and glycyl-leucine occur at 1395 cm^{-1} and 1410 cm^{-1}, respectively. The spectra of the complexes displayed a distinct medium intensity band at *ca.* 1380-1390 cm^{-1} region which may be assigned to ν_s(COO). The shifting of this band to lower frequency with the difference of ν_{as} - $\nu_s \approx 250$ cm^{-1} is typical of monodentate coordination of the carboxylate group via the O(carboxylate)

Table 4.2 *The structurally significant IR bands of peroxovanadate complexes* ***4.1-.4.4***

No.	Compound	IR bands					UV peak	
		ν(V=O)	ν(O-O)	ν_{as}(V-O_2)	ν_s(V-O_2)	ρ_r(H_2O)	nm	A(0.1 mM)
4.1	Na[VO(O_2)$_2$(gly-gly)(H_2O)].H_2O	966	871	632	539	725	325	0.57
4.2	K[VO(O_2)$_2$(gly-gly)(H_2O)].H_2O	968	870	630	542	725	323	0.54
4.3	Na[VO(O_2)$_2$(gly-leu)(H_2O)].H_2O	952	872	634	546	728	327	0.54
4.4	K[VO(O_2)$_2$(gly-leu)(H_2O)].H_2O	969	873	633	536	720	326	0.52

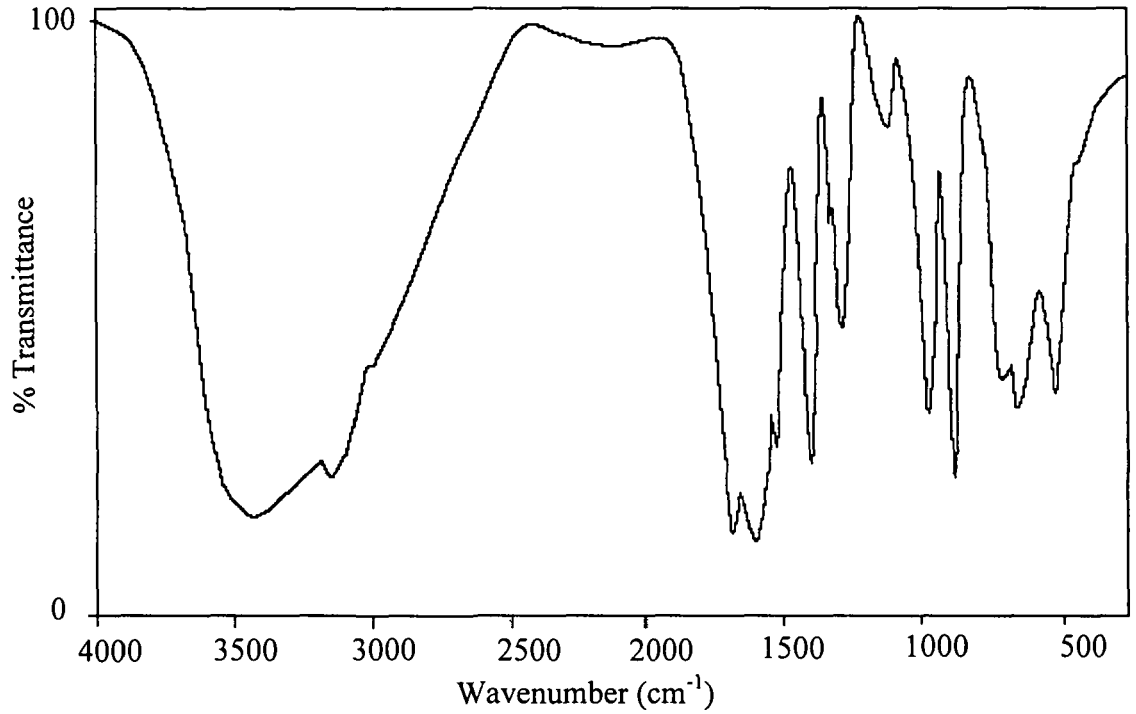


Fig. 4.1 IR spectrum of $Na[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$(**4.1**)

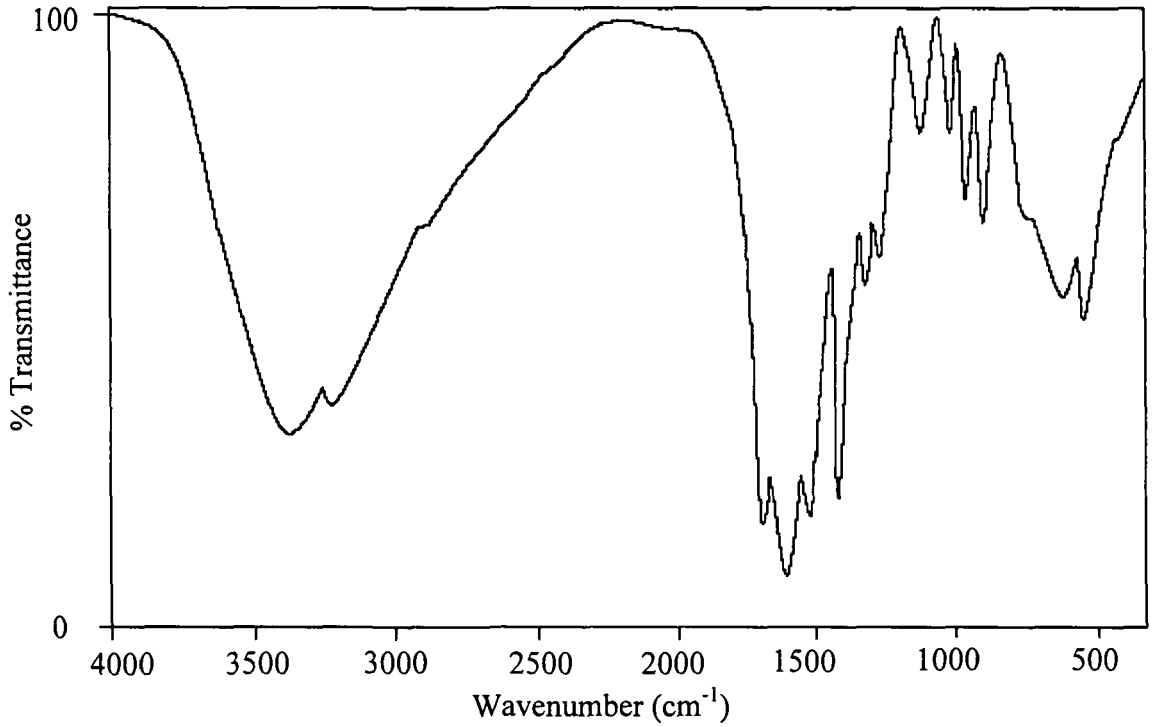


Fig.4.2 IR spectrum of of $Na[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$(**4.3**)

atom[57]. The N-H stretching modes of the peptide were observed in the 3300-3100 cm^{-1} regions as expected from the $-N^{+}H_3$ group. The rocking modes of $-N^{+}H_3$ appeared at *ca.*1130 and *ca.*1042 cm^{-1}. The broad and intense signal appearing at 3500-3400 cm^{-1}, characteristic of ν(OH) vibration suggested the presence of water. Owing to the presence of lattice water, IR spectral information on ν(OH) and δ(H-O-H) modes are not very significant in so far as the distinction between co-ordinated and lattice water are concerned. Fortunately, as in the case of molecular pW compounds **3.5** and **3.6**, here again the presence of co-ordinated water in each of the compounds was clearly suggested by the identification of a medium intensity signal at *ca.* 730 cm^{-1} attributable to rocking mode of water. IR spectral data thus attest to the existence of dipeptide ligands in the complexes **4.1-4.4** as zwitterions, unidentately co-ordinated to the metal centre through carboxylate group.

Electronic spectra of compounds **4.1** to **4.4** in aqueous solution displayed a weak intensity broad LMCT band (*Fig. 4.3 & 44*) originating from peroxide to vanadium π*-dσ transition [38]of at 320-330 nm (Table 4.2).

Spectral and chemical data provided evidence for the composition of the ligand sphere and the most likely mode of ligation to the metal ion. The proposed structures applicable to the complex species are shown schematically in *Fig. 4.5*.

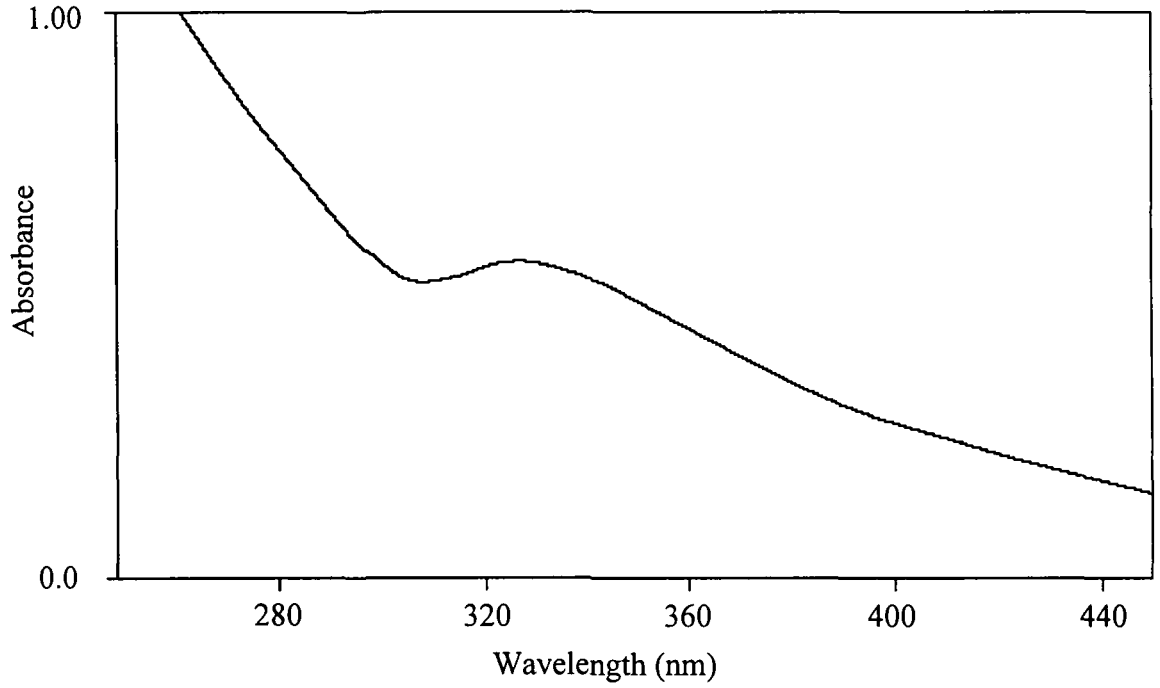


Fig. 4.3 UV spectrum of $Na[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$(**4.1**), (0.1mM)

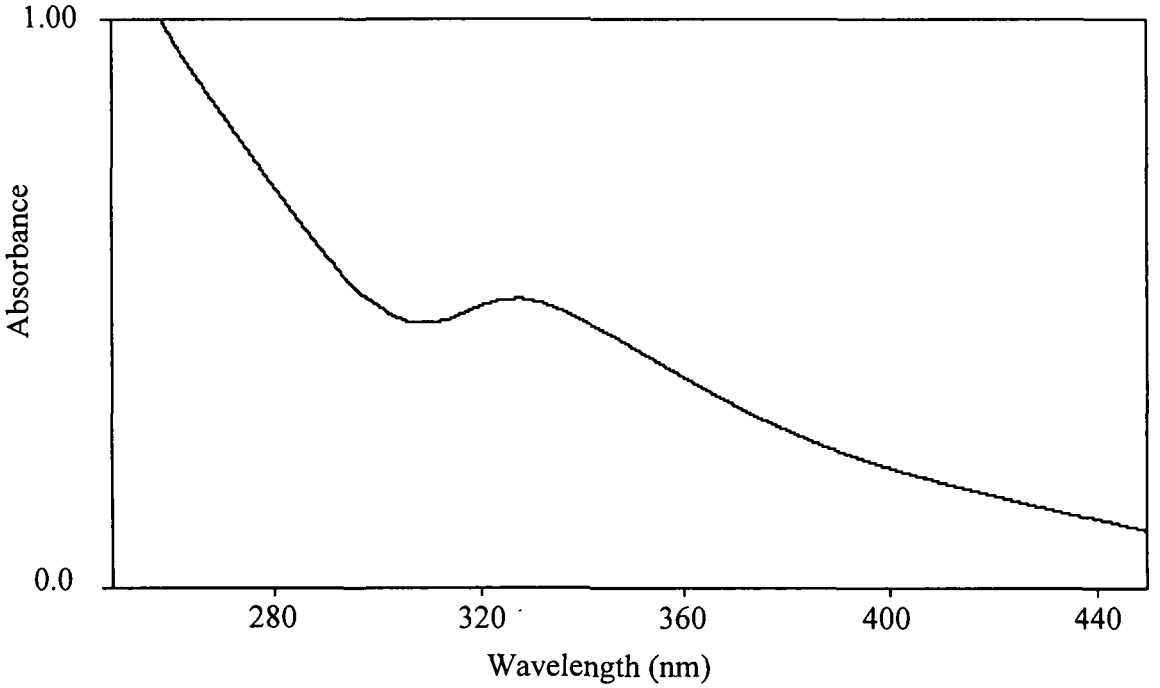
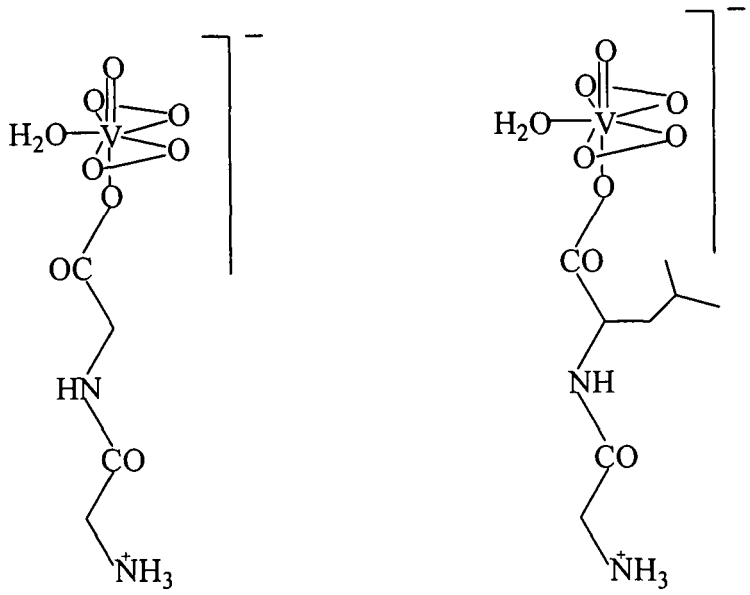


Fig. 4.4 UV spectrum of $Na[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$(**4.1**), (0.1mM)



(a) (b)

Fig. 4.5 A representation of proposed structure of the mononuclear peroxovanadate complex anions, (a) $[VO(O_2)_2(gly\text{-}gly)(H_2O)]^-$; (b) $[VO(O_2)_2(gly\text{-}leu)(H_2O)]^-$

4.3.2 Thermal analysis

Thermogravimetric analysis data of the compounds provided crucial information regarding composition of the compounds and their thermal stability. The thermograms of compounds **4.1** and **4.3** are presented in *Fig.4.6* and in *4.7,* respecively. It was apparent from the TGA data (Table 4.3) that the pV compounds, like the pW compounds reported in Chapter **3**, undergo multistage decomposition after the initial dehydration up to a final decomposition temperature of 550 ^{0}C. The first decomposition stage, observed between room temperature and temperature of 115 ^{0}C for compound **4.1** (118 ^{0}C for compound

Table 4.3 *Thermal decomposition of peroxovanadate (V) complexes* **4.1** *and* **4.3**

No	Compound	Temperature range(^{0}C)	% Weight loss					
			H_2O		O_2^{2-}		Total loss	
			Found	Calcd	Found	Calcd	Found	Calcd
1.	$Na[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$ (**4.1**)	30-115	11.2	11.6	-	-		
		115-200	-	-	18.9	19.9		
		up to 550					66.7	69.2
2.	$Na[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$ (**4.3**)	30-118	9.5	9.8	-	-		
		120-210	-	-	17.6	16.9		
		up to 550					74.7	75.4

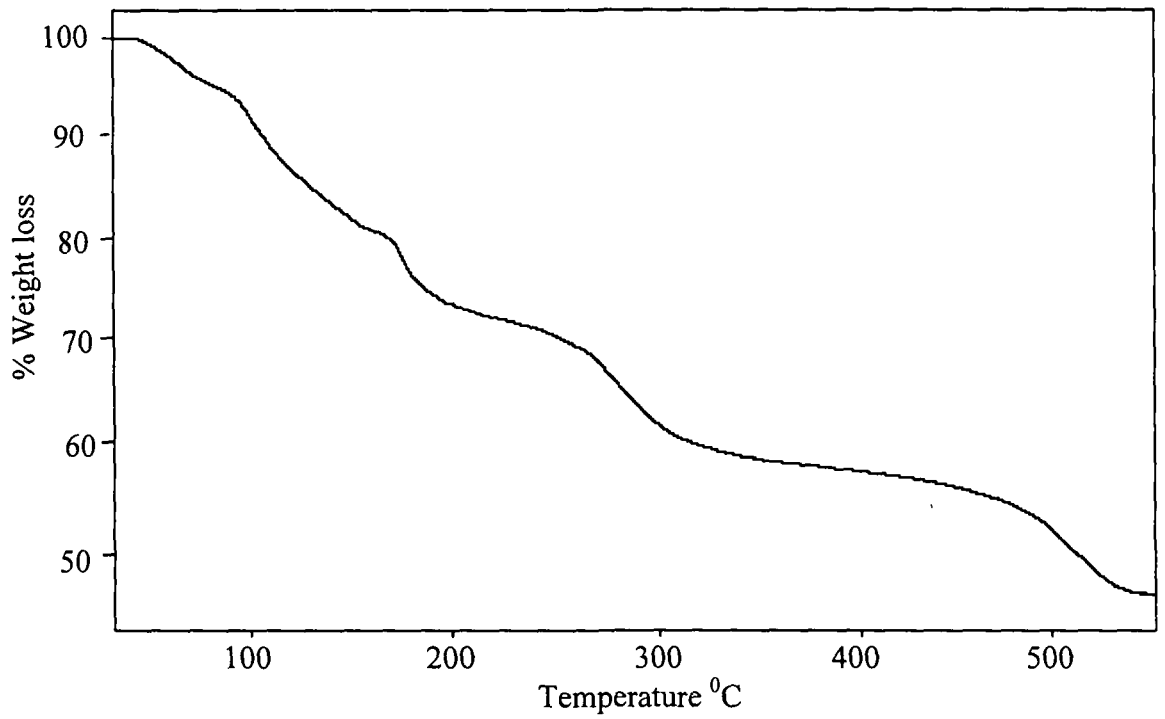


Fig. 4.6 TGA curve of $Na[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$(**4.1**)

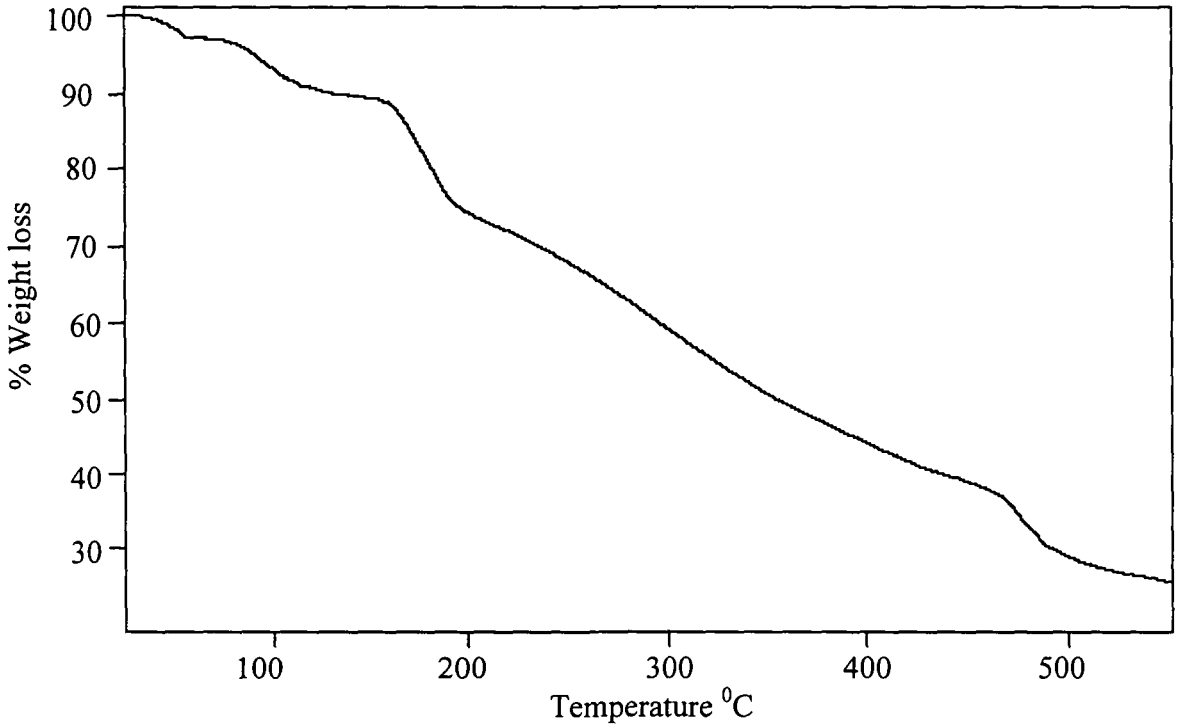


Fig. 4.7 TGA curve of $Na[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$(**4.3**)

4.3) is a two step one consistent with the liberation of two types of water molecules viz., outer sphere and co-ordinated. The corresponding observed weight loss of 11.6% (compound **4.1**) and 9.4 % (compound **4.3**) are in good agreement with the calculated values of 11.2 % and 9.5 % for the compounds **4.1** and **4.3**, respectively. The next decomposition stage is observed on heating up to a temperature of 200 ^{0}C (for compound **4.1**) and 160 ^{0}C (for compound **4.3**) with a corresponding weight loss attributable to loss of co-ordinated peroxo groups from the complexes. IR spectra of the decomposition products, isolated at this stage, showed the complete disappearance of peroxo group from the species. A close analogy has been observed between the TGA plots of pV compounds and the pW compounds containing glycyl-glycine and glycyl-leucine as co-ligands reported in Chapter **3**.

The complete loss of the components viz, water molecule, co-ordinated peroxide, and the dipeptide ligands was indicated by the thermograms on heating the compounds up to a final temperature of 550 ^{0}C. The total weight loss which occurred during the course of the overall decomposition process was in good agreement with the theoretically calculated values for each of the compounds **4.1** and **4.3** (Table 4.3). The residues remaining at this stage were confirmed to be oxo species of vanadium from their IR spectra, which displayed the characteristic ν(V=O) absorption. No bands attributable to peroxo or the dipeptide ligand of the original compound was observed in the IR spectra of the residues.

4.3.3 Nature and Stability of the complexes in solution

One of the primary interests of this study was to isolate pV complexes which would be stable under physiological conditions. Stability, lability and redox activity are key properties, which are critical for biochemical effect of vanadium compounds.

The stability of the compounds **4.1- 4.4** in aqueous solution of pH *ca.* 5, which is the natural pH of the solution of the compounds in water, as well as in buffered solutions of a range of pH values viz., 3.6, 7.0 and 8.0 were tested by methods described in Chapter **3**. It has been evident from the data that the compounds remained fairly stable in solution without losing their peroxide content, which has also been reflected in their electronic spectra by the unchanged position and intensity of the band at 310-320 nm measured at specific time intervals, for a period of 12 h. Moreover, the results of conductance measurements obtained after a period of 12 h, were practically similar to those recorded on freshly prepared solutions. These observations attest to the stability of the complexes in solution. *Fig. 5.1* of Chapter **5** demonstrate that the compounds **4.1** and **4.3**, used as representatives, are stable in solution of pH 5.5 as well as at pH 7.0.

4.3.4 ^{51}V-NMR spectral analysis of aqueous solution of compounds

Further information regarding the nature and stability of the complexes in solution was derived from ^{51}V-NMR studies on compounds **4.1** and **4.3**. The spectrum of each of the tiltle complexes displayed a single major peak at –705 ppm indicating the presence of ligated diperoxovanadate as the predominant species. The assignment of the peaks in the

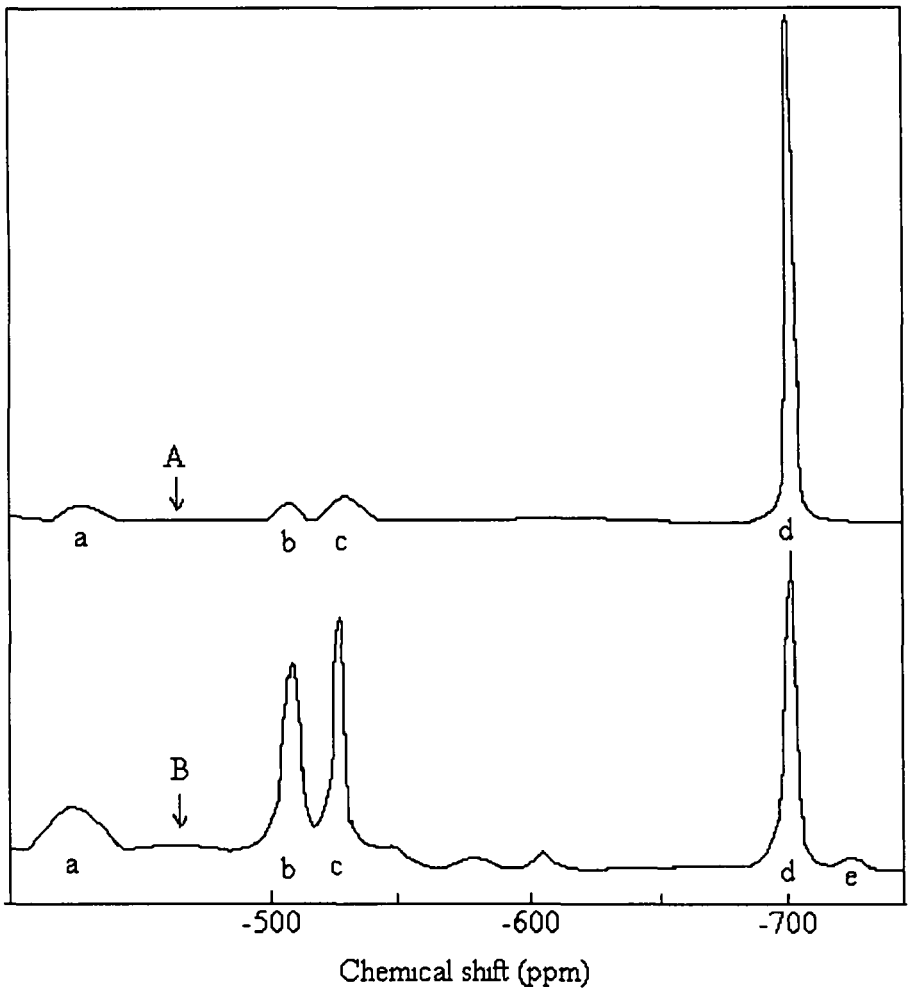


Fig. 4.8 ^{51}V-NMR spectrum of aqueous solution of peroxovanadate complex **4.1** (A). Solutions of the compounds were obtained by adding the solids to water. (B) Spectrum recorded after ca. 12 h of solution preparation. Identification of the peaks: a, b and c, the three peaks (2:2:1) of decavanadate (V_{10}); d, free vanadate (V_1); e, ligated diperoxovanadate.

present study was on the basis of available data[39,58-60]. The small variations from the reported chemical shift values may be due to the presence of the co-ordinated ligands and to variation of pH. No notable change in the pattern of the spectra or position of the signal was observed for over a period of *ca.*12 h. Stability of the compounds in solution during this period was thus implicit. However, in the spectra of the complexes, recorded after *ca.* 14 h of preparation of solution, three additional weak intensity peaks appeared at - 427, -509 and -527 ppm with intensity ratio of 1:2:2 indicating the presence of traces of vanadate.

4.4 DISCUSSION

The complexity involved in the chemistry of V-H_2O_2 system is evident from the different colour reactions observed with small variation of pH. In a solution of vanadate and excess H_2O_2 at a pH >5, formation of a yellow diperoxovanadate (DPV) species is known to predominate[35,38]. In the present study, the strategically maintained pH of *ca.* 5 was found to be conducive for the successful synthesis of the diperoxvanadate compounds **4.1** – **4.4**. It is pertinent to mention here that maintenance of acidic pH of *ca.* 3 was one of the essential requirements for the syntheses of a series of peptide containing μ-peroxo divanadates reported previously by us[44-46]. These observations underscore further the importance of pH for the desired synthesis of peroxo-vanadium compounds.

One of the notable common features between peptide coordinated dinuclear and mononuclear pW compounds **3.3-3.6** and pV compounds **4.1-4.4** is the occurrence of the

ancillary ligands, gly-gly and gly-leu in their unidentate zwitterionic forms. As mentioned in the previous Chapter, the presence of several donor sites in the amino acids and peptides make them co-ordinatively versatile as ligands. Although the bi or tridentate co-ordination is more common in amino acid or peptide containing complexes[55,56, 61,62] however, monodentate co-ordination through a carboxylate group of the amino acid or peptide zwitterions is not unprecedented [43-45,63]. It is notable that in a structurally characterized monoperoxo-vanadate compound reported previously [46] glycyl-glycine was observed to be co-ordinated in a tridentate fashion involving carboxylate, deprotonated N-amide and $-NH_2$ groups. This compound was isolated from a solution of pH 6.8. In the present case, the pH value of *ca.* 5 of the reaction medium is unlikely to allow deprotonation of the amide group and thereby probably limits co-ordination to the metal centres through carboxylate group of the peptide zwitterion.

It is noteworthy that the compounds retain their structural identity in solution for a reasonable period of time. Interestingly, the stability of these pV compounds in solution was found to be comparable to that of the amino acid and peptide containing pW compounds **3.1-3.6**. The enhanced stability of the monomeric peptide containing pV compounds, compared to the triperoxvanadates with a μ-peroxo group reported earlier, may be attributed to the presence of DPV moiety in these complexes with exclusively side-on bound peroxo groups. The results are consistent with earlier suggestion that the monomeric DPV complexes possess much higher stability in solution as compared to the peroxo-bridged dinuclear compounds in similar co-ligand environment.

The present work thus afforded a set of peroxo derivatives of vanadium, which contain species familiar to bioenvironment as ancillary ligand. A distinctive feature of the

compounds is their high stability in solution of a wide range of pH values including acidic as well as physiological pH. Results of investigation on some of the biochemical properties of the title compounds are reported in Chapter **5** of the thesis.

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CHAPTER 5

Peroxo compounds of vanadium(V) and tungsten(VI) as potent inhibitors of alkaline phosphatase activity: their interaction with glutathione and catalase*

5.1 INTRODUCTION

With the discovery of several tungstoenzymes in recent years biological relevance of tungsten and its compounds has finally been recognized[1,2]. Moreover, recent reports on biochemical activity of the compounds of tungsten such as their antiviral activity and affinity to inhibit a number of enzyme functions[3,4] including hydrolysis of phosphoprotiens[5-9], came as exciting contributions to the current knowledge of biological importance of the metal and its compounds. These fascinating findings and the observations that tungstate and peroxotungstate (pW) formed in a solution of W-H_2O_2, like vanadate and peroxovanadate (pV), were capable of stimulating most of the insulin bioeffects in rat adipocytes[9] led to a revival of interest in peroxo-tungsten systems.

It is well established that vanadate and pV inhibit a variety of enzymes which catalyze phosphoryl transfer reactions[10] and exhibit insulin-like action[11-23].Peroxovanadate is reported to be far more potent in facilitating the metabolic effect of insulin than vanadate. The exact mechanism by which peroxotungstate or peroxovanadate mimic the action of insulin or inhibit enzyme function is yet to be fully understood [9,21-23]. However, a definite

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correlation has been established between abilities of vanadate and pV complexes to inhibit protein phosphatases, their abilities to promote activation of insulin receptor, and their *in vivo* insulin mimetic activities[21-23] . In order to gain an insight into the role of vanadium in bio-processes a variety of synthetic peroxo vanadium compounds with different ancillary ligands have been synthesized and studied as biomimetic models[24-27]. Much research has also been performed on the potential usage of compounds of vanadium as therapeutic antidiabetic agents[24-27], as well as their significant affinity as phosphatase inhibitors[21-23]. However, as already mentioned in the introductory Chapter, most of the synthetic pV compounds tested for their various biochemical effects suffer from the disadvantage of being unstable in solution which limits their pharmacological potential[23,24,26]. Consequently, efforts to generate stable pV systems possessing appropriate bio-relevat characteristics continues unabated.

It is notable in this context that peroxotungstates, formed in a solution of W-H_2O_2 not only exhibited insuline-like behaviour but were also found to be stable in solution of a wide range of pH values[9]. Most importantly, W has been known to be considerably less toxic compared to V. Surprisingly, despite these important findings on favorable properties of tungstate and pW species, we are yet to come across reports dealing with screening of discreet synthetic pW complexes for their biochemical properties such as their insulin like activity or their effect on activation or inhibition of different enzyme functions including phosphatases[3]. The importance of enzyme inhibition as a mode of action for inorganic drugs is being recognized in recent years and is an area needing exploration[3].

As a direct sequel to our work on peroxometallates, described in Chapters **3** and **4** of the thesis, which afforded reasonably stable peptide containing peroxo compounds of V(V) and W(VI), we deemed it worthwhile to examine some of the biochemically important

aspects of the newly synthesized compounds. Reported in this Chapter are the results of studies on the redox activity of the compounds $A_2[W_2O_3(O_2)_4(cystine)].4.H_2O$ [A = Na (**3.1**) or K (**3.2**)], $Na_2[W_2O_3(O_2)_4(dipeptide)_2].3H_2O$ [dipeptide = glycyl-glycine (**3.3**) and glycyl-leucine, (**3.4**)] and $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$ [dipeptide = glycyl-glycine (**3.5**) or glycyl-leucine (**3.6**)] with respect to reduced glutathione (GSH), their interaction with catalse and effect of the compounds on the activity of alkaline phosphatase. These peroxo compounds of the two d^0 metals viz. V(V) and W(VI) with similar ancillary ligands in their co-ordination spheres provided a scope to draw comparisons on the specific properties investigated.

5.2 EXPERIMENTAL SECTION

5.2.1 Spectroscopic measurements

Spectroscopic determinations of the initial rate of ALP catalyzed hydrolysis of pNPP and oxidations of GSH were carried out in a Cary model Bio 100 spectrophotometer, equipped with a peltier controlled constant temperature cell. The absorbance values were denoted as e.g. A_{405} at the wavelength indicated.

5.2.2 Effect of catalase on the complexes

The effect of catalase on complexes was studied by estimating the peroxide content of the compounds in a solution containing catalase at specified time intervals volumetrically, as well as spectrophotometrically by methods described in Chapters **2** and **3**(*Fig. 5.1&5.2*).

The test solution contained phosphate buffer (50 mM, pH 7.0) and catalase (40 μg/ml). The volume of the reaction solution was kept at 25 ml. The solution was incubated at 30 ^{0}C. The compound was then added to the test solution and aliquots of 5ml were pipetted out and titrated for peroxide content after stopping the reaction by adding it to cold sulfuric acid (0.7 M, 100 ml) at time 5, 10, 15, 20, 25 and 40 min of starting the reaction. Three concentrations of peroxo compounds (0.05, 0.1, 0.2 mM) were tested.

5.2.3 Measurement of redox activity in solution

To a reaction mixture containing GSH (80 μM) and phosphate buffer (50 mM, pH 7.0) a measured amount of aliquot from solution of the synthesized compound (1mM) was added. Following incubation of 10 min, DTNB (160 μM) was added to the solution and the change in absorbance at 412 nm was determined in order to measure the GSH remaining in solution by the method of Ellman[28] using molar extinction of ε_{412} =13,600. Measurements were done by using different concentrations of the compounds, 5.0, 10.0 μM, in triplicate under same assay conditions.

5.2.4 Measurement of alkaline phosphatase activity

Phosphatase activity was assayed spectrophotometrically by using p-nitrophenyl phosphate (p-NPP) as a substrate. The continuous production of p-nitrophenol (p-NP) was determined at 30 ^{0}C by measuring absorbance at 405nm in a reaction mixture containing ALP from rabbit intestine (3.3 μg protein/ ml), p-NPP (1 mM) in incubation buffer (25 mM

glycine + 2 mM $MgCl_2$, pH 10.0). The initial reaction rates were obtained by starting the reaction by adding ALP to the reaction solution, which was pre-incubated for 5 min. The initial reaction rate of p-NPP hydrolysis in the absence of the inhibitors, V_0 was determined which was used as control. The effects of peroxotungstate, peroxovanadate and other inhibitors were assessed by adding different concentrations (10 -100 μM) of each species in the ALP assay. The V_i was obtained as the rate of p-NPP hydrolysis in the presence of variable concentrations of inhibitors under similar experimental conditions. The V_0/V_i ratios were calculated from these values. The concentrations tested for the compounds and each of the other inhibitors were 10, 20, 30, 40, 50 and 75 μM. Solutions of V(V)-H_2O_2 and W(VI)-H_2O_2 of equivalent concentrations were prepared by adding required amount of aliquot to the test solution from a freshly prepared stock solution containing vanadate (1 mM) or tungstate (1 mM) and H_2O_2 (2 mM). The IC_{50} values were graphically determined as the half-maximal inhibitory concentration of the inhibitor species giving 50% inhibition. All the assays were performed in triplicate. The data in figures are presented as the means ± SE from three separate experiments.

5.3 RESULTS AND INTERPRETATION

5.3.1 Effect of catalase on peroxovanadium and peroxtungsten compounds

Keeping in view our goal of studying some of the biochemically relevant properties of the complexes, we considered it important to examine the effect of the enzyme catalase, the ubiquitous enzyme that catalyze the breakdown of H_2O_2 formed during oxidative

processes in the intercellular peroxisomes. Addition of catalase, to phosphate buffered solution of H_2O_2 released a half-equivalent (molecular basis) of oxygen, as expected from disproportionation reaction, which will be completed in less than 2 min[29]. On incubation with catalase, each of the peroxotungsten compounds **3.1-3.6** and peroxovanadates **4.1- 4.4**, which was otherwise ascertained to be stable in solution of a wide range of pH values, was found to be degraded slowly with the loss of peroxide and concomitant release of oxygen (Table 5.1).

Table 5.1. *Catalase dependent oxygen release from pW and pV compounds*

Sl. No.	Compounds		Conc(mM)	Loss of peroxide	
				μM /min.	Total (μM)
1.	$Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$	**(3.1)**	0.1	22.2	37.4
2.	$K_2[W_2O_3(O_2)_4(cystine)].4H_2O$	**(3.2)**	0.1	20.4	38.3
3.	$Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$	**(3.3)**	0.1	21.3	39.2
4.	$Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$	**(3.4)**	0.1	24.1	39.4
5.	$[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$	**(3.5)**	0.1	6.8	19.1
6.	$[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$	**(3.6)**	0.1	8.7	19.7
7.	$Na[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$	**(4.1)**	0.1	17.0	19.6
8.	$K[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$	**(4.2)**	0.1	18.2	19.2
9.	$Na[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$	**(4.3)**	0.1	20.0	19.9
10.	$K[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$	**(4.4)**	0.1	19.5	19.8

Total peroxide loss from each of the pV compound solutions of 0.1 mM concentration tested was recorded to be *ca.* 0.2 mM for the compounds, (Table 5.1) indicating a ratio of 1:2 for peroxide: peroxovanadate compounds which is in excellent agreement with the estimated peroxide content of the compounds. The extent of degradation of the peptide containing oxodiperoxovanadate complexes **4.1-4.4** under the effect of catalase action was found to be comparable to that of diperoxovanadate (DPV)[29], indicating their similarity with respect to number of peroxide. However, their rates of degradation were observed to be slower than DPV (26 μM/min from a solution of 0.1 mM) indicating a relatively greater resistance of the compounds to catalase compared to DPV. The effect of catalase on the complexes is shown in *Fig. 5.1*, tested with compounds **4.1** as representatives.

Each of the dinuclear tetraperoxotungsten compounds 3.1 -3.4 were found to be degraded in presence of the enzyme with rate of peroxide loss varying within the range of 20-24 μM/min from a solution of 0.1 mM which contained *ca.* 0.4 mM of peroxide (*Fig.5.2*). The rates of degradative loss of peroxide from the monomeric diperoxotungsten compounds under analogous condition are presented in Table 5.1 and shown in *Fig.5.2*.

Total peroxide loss from the dinuclear and mononuclear pW compound solutions of 0.1 mM concentration tested was recorded to be *ca.* 0.4, and *ca.* 0.2 mM, respectively (Table 5.1). The data thus suggested a ratio of approximately 1:4 for peroxide: dinuclear complex (compounds **3.1** – **3.4**) and 1:2 for mononuclear pW compounds (**3.6** and **3.6**), which are consistent with the estimated peroxide content in these compounds.

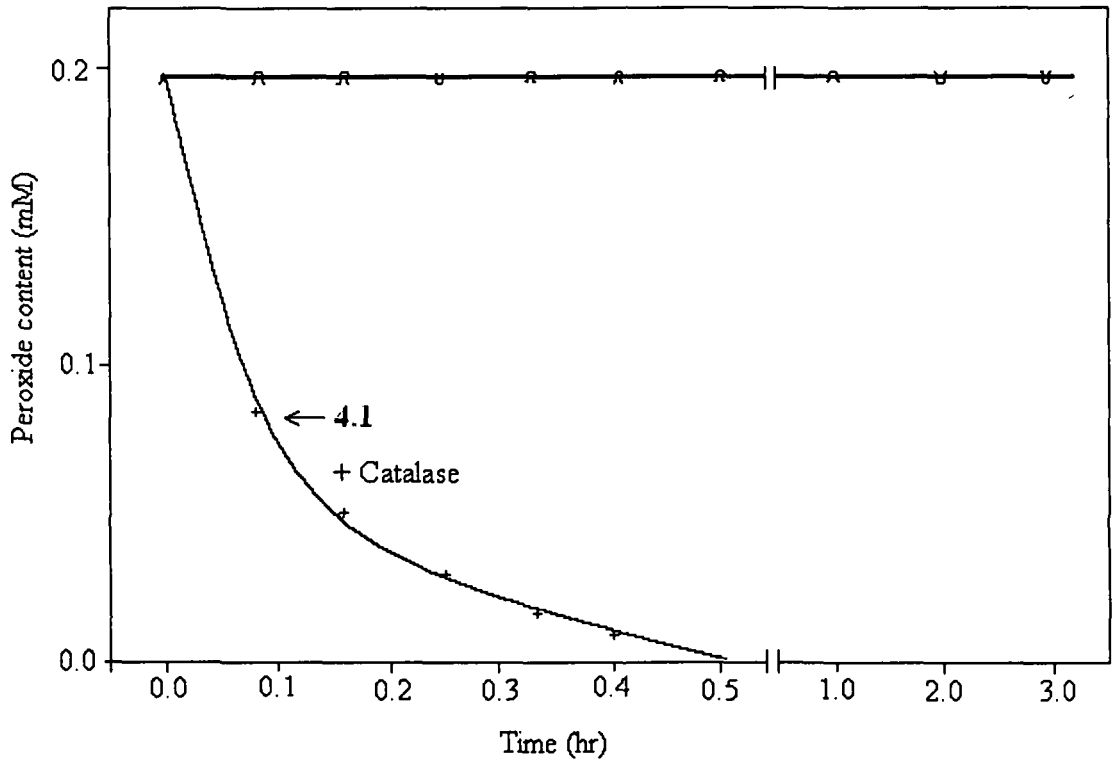


Fig. 5.1. Stability of the pV complexes at different pH values, effect of catalase on compound **4.1**. □: Compound solution in phosphate buffer (50 mM, pH 7.0). ×: Solution of complexes in phosphate buffer (pH 8.0). +: Effect of catalase. The test solution contained phosphate buffer (50 mM, pH 7.0) and the catalase (40μg /mL) which was incubated at 30 ^{0}C for 5 min. Compounds (0.1 mM) were then added to the reaction solution and aliquots were drawn at indicated time points and loss in peroxide content was determined

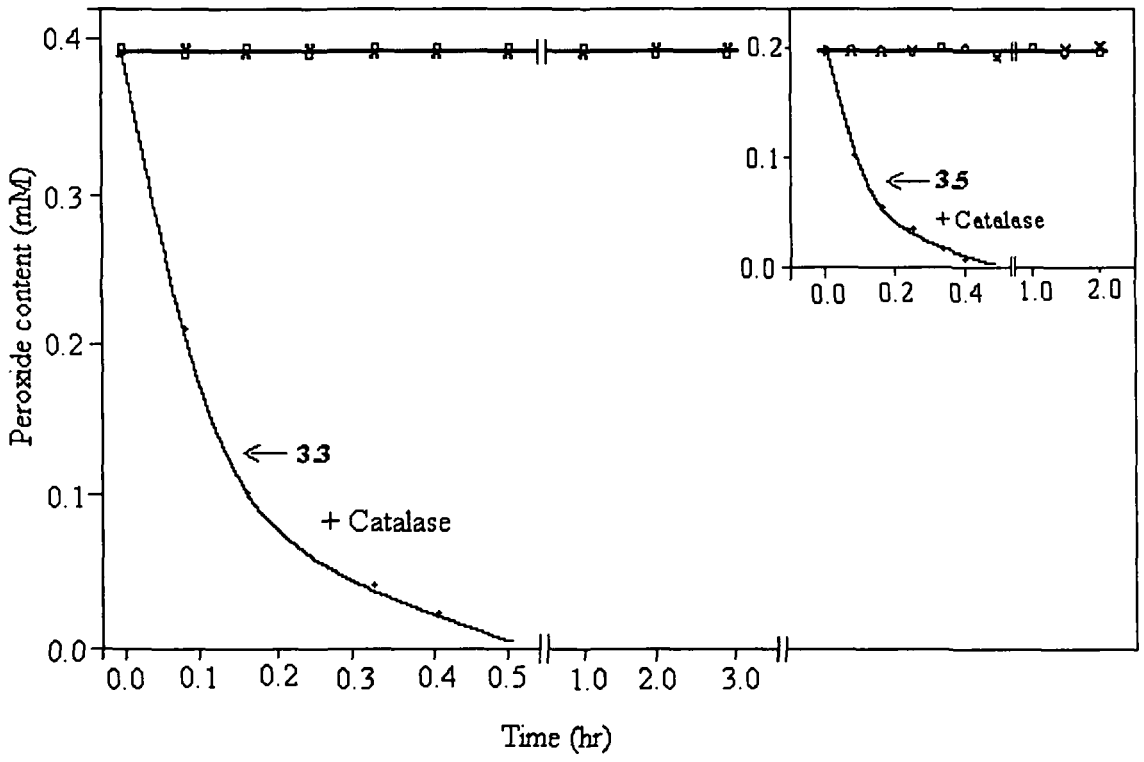


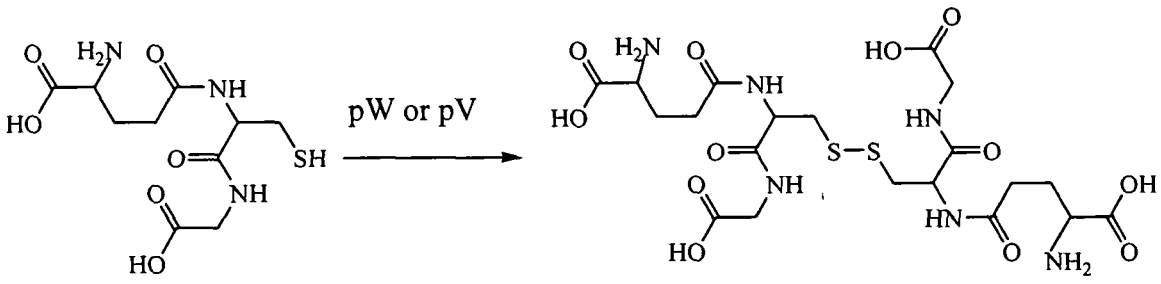
Fig. 5.2. Stability of the pW complexes at different pH values, effect of catalase on compound **3.3** and **3.5** (shown in inset). □: Compound solution in phosphate buffer (50 mM, pH 7.0). ×: Solution of complexes in phosphate buffer (pH 8.0). +: Effect of catalase. The test solution was prepared as described under *Fig. 5.1.* Compounds (0.1 mM) were then added to the reaction solution and aliquots were drawn at indicated time points and loss in peroxide content was determined

Under the effect of catalase the rate of degradation of H_2O_2 with the release of oxygen was reported to be 430 μM /min[29] from a solution of 0.1 mM concentration. Thus the rate of H_2O_2 degradation is much higher than the rates of degradation observed for the title compounds under similar reaction conditions. It is therefore evident that the synthesized pV and pW complexes are at least 20-80 times weaker as substrates to catalase compared to H_2O_2, its natural substrate. It is also notable that among the monomeric pV and pW compounds tested, pW compounds were found to exhibit greater resistance to catalase.

5.3.2 Oxidation of glutathione (GSH) by pV and pW complexes

Oxidizing capacity of permetalloxides with respect to reduced GSH, were tested by the method of Ellman[28]. The method involves determination of GSH concentration by the kinetic assay wherein GSH is continuously oxidized to GSSG by 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) which in turn is reduced to the 412 nm absorbing coloured product, 5-thionitrobenzoate (TNB).

Each of the monomeric pV and pW compounds as well as dinuclear peroxo tungsten compounds tested, have been found to be oxidizing agent with respect to reduced glutathione(GSH). The amounts of GSH oxidized at two different concentrations of 5 and 10 μM of the pW compounds **3.1, 3.3** and **3.5**, are presented in *Fig. 5.3*. If all the four peroxo groups of the dimeric compounds **(3.1–3.4)** were active in oxidation, 8 moles of GSH to GSSG per mole of the compound should have been oxidized. Similarly, in view of the presence of two peroxo groups it is expected that compounds **3.5** and **3.6** would oxidize 4



GSH **GSSG**

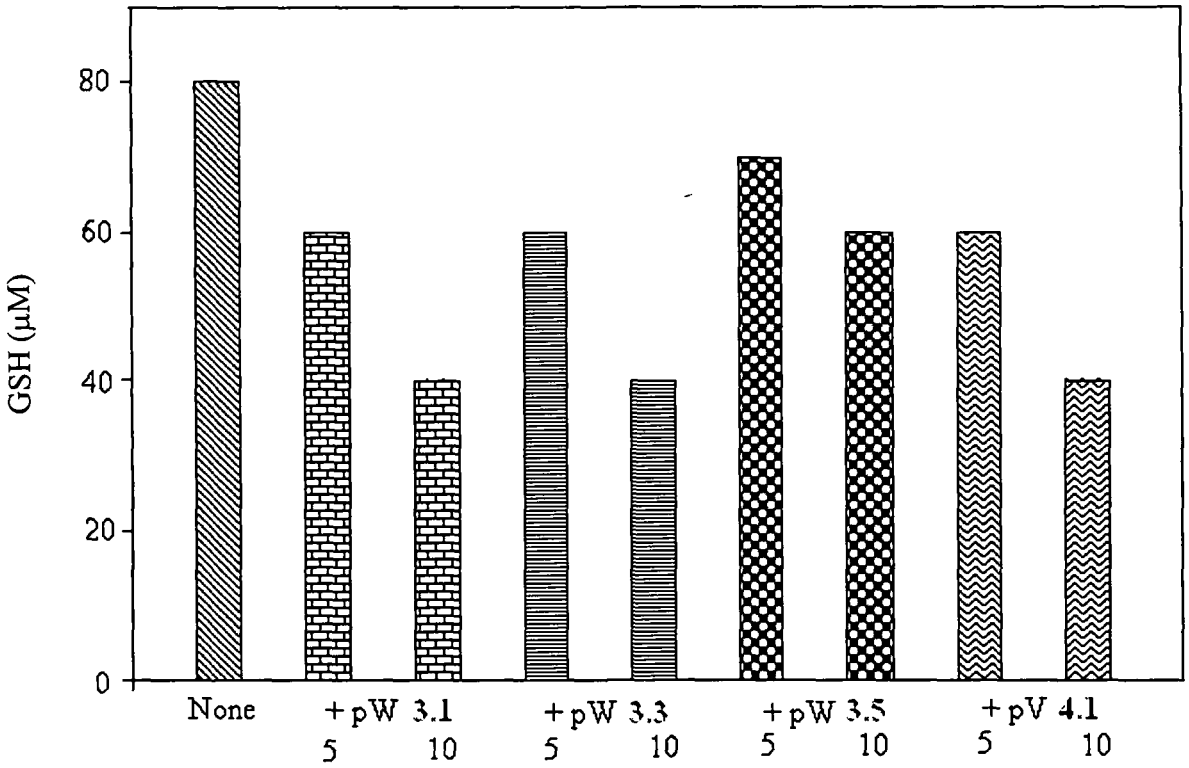


Fig. 5.3. Oxidation of GSH by dinuclear pW (**3.1&3.3**), monomeric pW (**3.5**) and mononuclear pV (**4.1**) compounds. The reaction mixture contained GSH (80 μM) and phosphate buffer (50 mM, pH 7.0). A measured amount of the synthesized compound was added. Measurements were done by using two different concentrations of the compounds, 5.0 and 10.0 μM in triplicate under same assay conditions. Following incubation of 10 min, DTNB (160 μM) was added to the solution and the change in absorbance at 412 nm was determined in order to measure the GSH remaining in solution.

Table 5.2 *Oxidation of GSH by pW and pV compounds*

Sl.no. Compounds	Concentration (μM)	GSH oxidized (μM)
1. $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$(**3.1**)	5	20.0
	10	39.8
2. $K_2[W_2O_3(O_2)_4(cystine)].4H_2O$(**3.2**)	5	19.6
	10	39.7
3. $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$ (**3.3**)	5	19.9
	10	40.0
4. $Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$ (**3.4**)	5	20.0
	10	39.3
5. $[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$(**3.5**)	5	9.8
	10	20.0
6. $[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$ (**3.6**)	5	9.6
	10	19.7
7. $Na[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$ (**4.1**)	5	20.0
	10	38.8
8. $K[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$ (**4.2**)	5	19.5
	10	40.0
9. Na $[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$ (**4.3**)	5	19.4
	10	38.9
10. $K[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$ (**4.4**)	5	19.6
	10	39.2

moles GSH. However, the consistently observed stoichiometry of 4: 1 for GSH oxidized: dinuclear pW compounds (**3.1** or **3.3**) and 2:1 for GSH: mononuclear pW compound (**3.5** or **3.6**) cause us to infer that only one of the peroxo groups of a diperoxotungsten, $WO(O_2)_2$ moiety of the monomeric compounds or two of the peroxo groups of the oxo-bridged tetraperoxo tungsten species, $[\{WO(O_2)_2\}_2O]$ of compounds **3.1** and **3.3** would probably be active in GSH oxidation. Significantly, in contrast to the above observation, the pV compounds **4.1-4.4** stoichiometrically oxidized GSH to GSSG (*Fig. 5.3*) with a molar ratio of 4:1 for GSH oxidized to pV compound as anticipated from the presence of two peroxo groups in these compounds (Table5.2)

5.3.3 Effect on alkaline phosphatase activity of the pW and pV compounds

Using rabbit intestine alkaline phophatase, employing the established enzyme assay system and p-NPP as substrate, the effect of the newly synthesized peroxo-metal complexes upon ALP activity was systematically investigated. The dose dependent effects of the pV complexes, **4.1** and **4.3** and mononuclear and dinuclear peroxo tungstate complexes, **3.1-3.6** in comparison to the effects induced separately by the corresponding free amino acid or dipeptide ligand, vanadate and tungstate, as well as peroxovanadate and peroxotungstate generated in solution are presented in *Fig. 5.4 and 5.5*.

To quantify the inhibitory potential of the molecules, the half-maximal inhibitory concentration (IC_{50}) for each inhibitor which gave rise to a 50% suppression of the original enzyme activity was determined (Table 5.3). Moreover, the enzymatic rate ratios of V_0/Vi

Table 5.3 *Half-maximal inhibitory concentration (IC_{50}) values of the pW and pV compounds and other inhibitors against ALP*

Inhibitor		IC_{50} (μM)
1. $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$	**(3.1)**	8.2
2. $K_2[W_2O_3(O_2)_4(cystine)].4H_2O$	**(3.2)**	8.0
3. $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$	**(3.3)**	10.29
4. $Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$	**(3.4)**	12.67
5. $[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$	**(3.5)**	15.84
6. $[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$	**(3.6)**	19.80
7. Na $[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$	**(4.1)**	11.88
8. K $[VO(O_2)_2(gly\text{-}gly)(H_2O)].H_2O$	**(4.2)**	12.45
9. Na $[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$	**(4.3)**	15.07
10. K $[VO(O_2)_2(gly\text{-}leu)(H_2O)].H_2O$	**(4.4)**	16.27
11. Tungstate		31.68
12. Tungstate/H_2O_2		25.34
13. Vanadate		30.09
14. Vanadate/ H_2O_2		24.17
15. Glycyl-glycine		-
16. Glycyl-leucine		-

Note: The ALP catalyzed rates of hydrolysis of p-NPP at pH 10.0 were determined at 30 ^{0}C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/ml), p-NPP (1 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) in the presence of stated concentrations of the inhibitors (*Fig.5.4 & 5.5*).

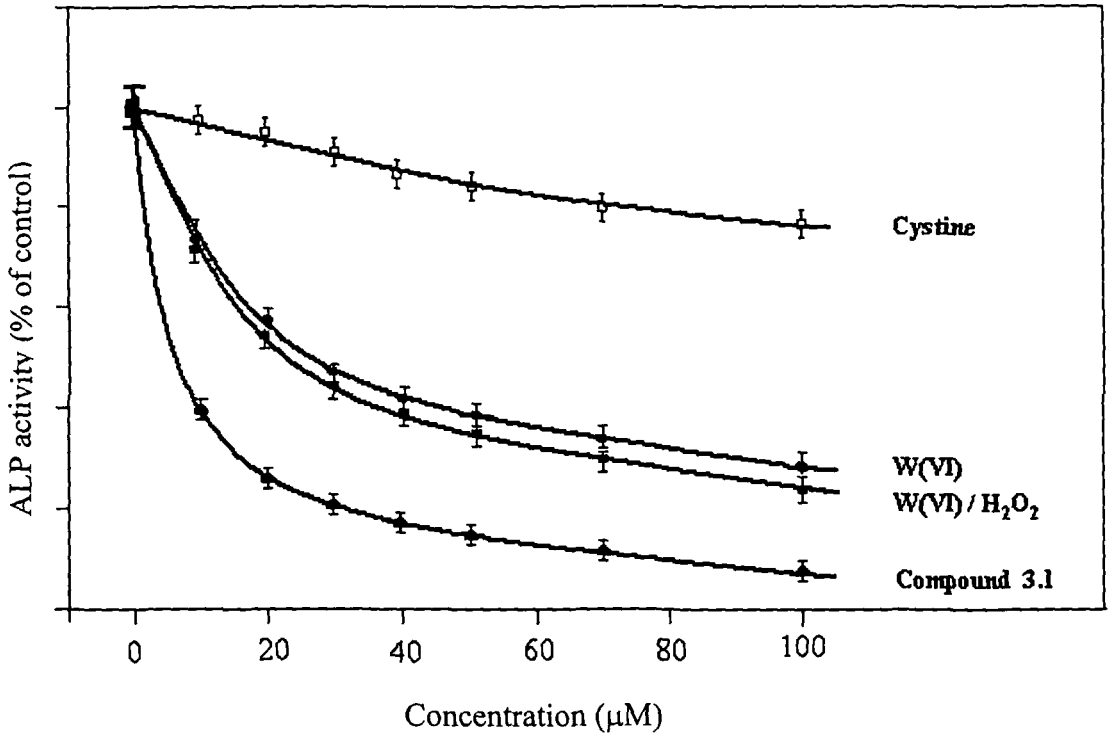


Fig. 5.4. Effect of pW compound **3.1**, tungstate, cystine and W(VI)/H_2O_2 species on activity of ALP from rabbit intestine. The ALP catalyzed rates of hydrolysis of p-NPP at pH 10.0 were determined at 30 ^{0}C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/ml), p-NPP (1 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) in the absence or presence of stated concentrations of the inhibitors. Effects of the additions are represented as the percent values (rounded to integers) of control (Δp-NPP = 0.96 μM/min). The data are presented as the means ± SE from three separate experiments.

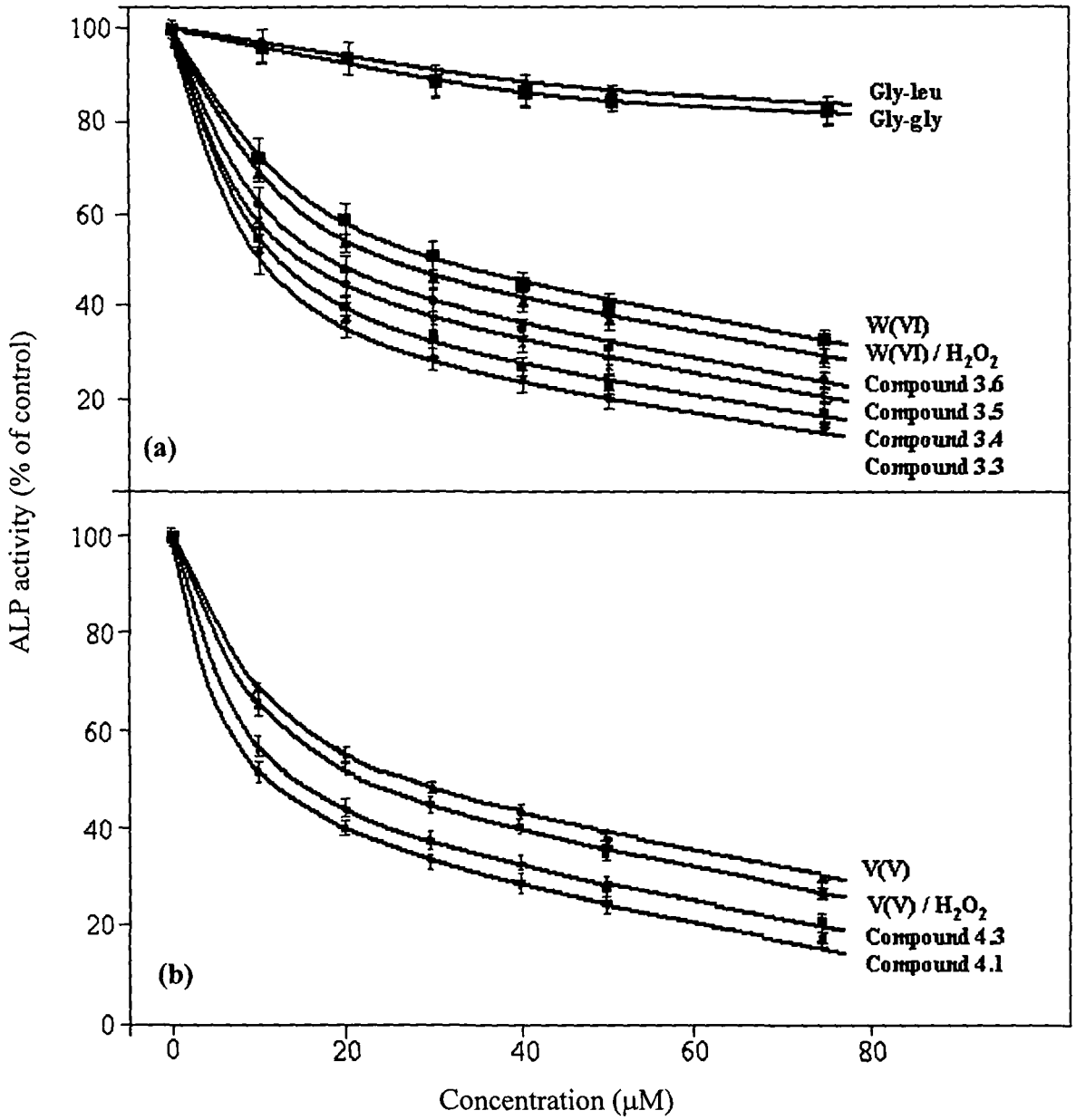


Fig. 5.5. Effect of **(a)** pW compounds **3.3 – 3.6**, tungstate, W/H_2O_2 and **(b)** pV compounds **4.1** and **4.3,** gly-gly, gly-leu, vanadate, $V(V)/H_2O_2$ species on activity of ALP from rabbit intestine. The assay of the reaction medium was similar to that described under *Fig. 5.4*.

where V_0 is the uninhibited rate and V_i is the rate of the enzymatic reaction inhibited by the complexes and other species have also been determined. The V_0/V_i ratio was found to be directly proportional to the different compound concentrations. The results obtained can be seen in *Fig. 5.6 and 5.7*. It is evident from the data derived from Vo/Vi relationships and

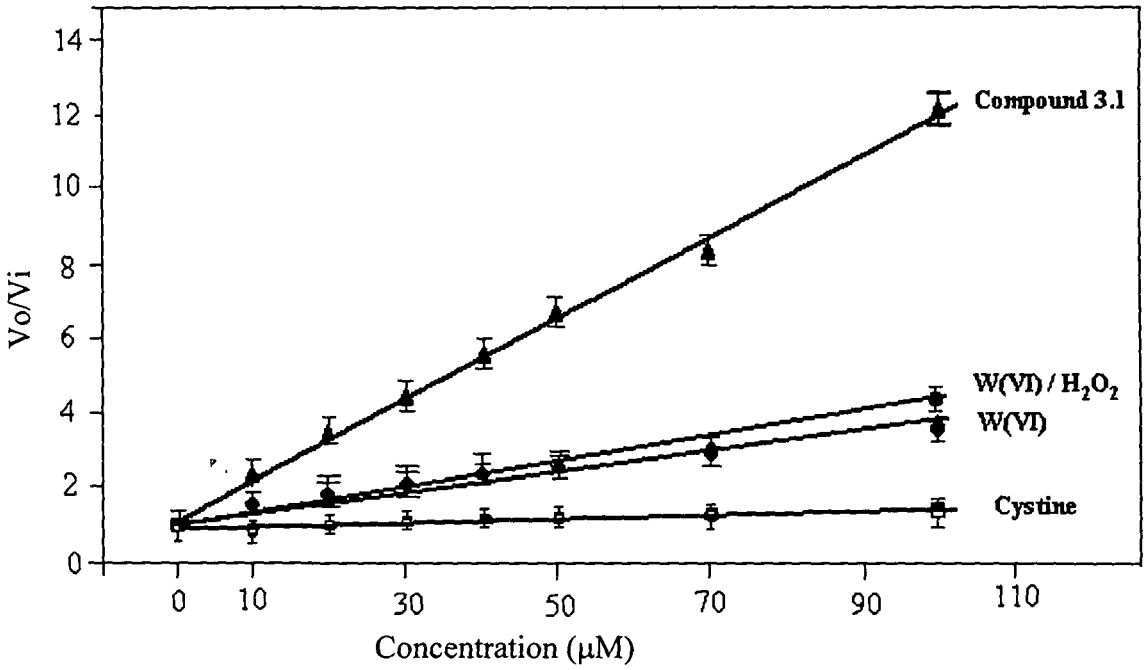


Fig. 5.6 V_0/V_i ratios for the inhibition of compound **3.1,** W(VI) , cystine and W(VI)/H_2O_2 species in the alkaline phosphatase catalyzed hydrolysis of p-NPP. The reaction mixture contained glycine buffer (25mM glycine + 2mM $MgCl_2$, pH 10.0)and p-NPP(1mM). The reaction was started by adding ALP (3.3 μg/ml) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the absence of the inhibitors, V_0, was obtained. The inhibited rates of hydrolysis, V_i, were determined as above and in the presence of stated concentrations of inhibitors. The values are expressed as means ± SE from three separate experiments.

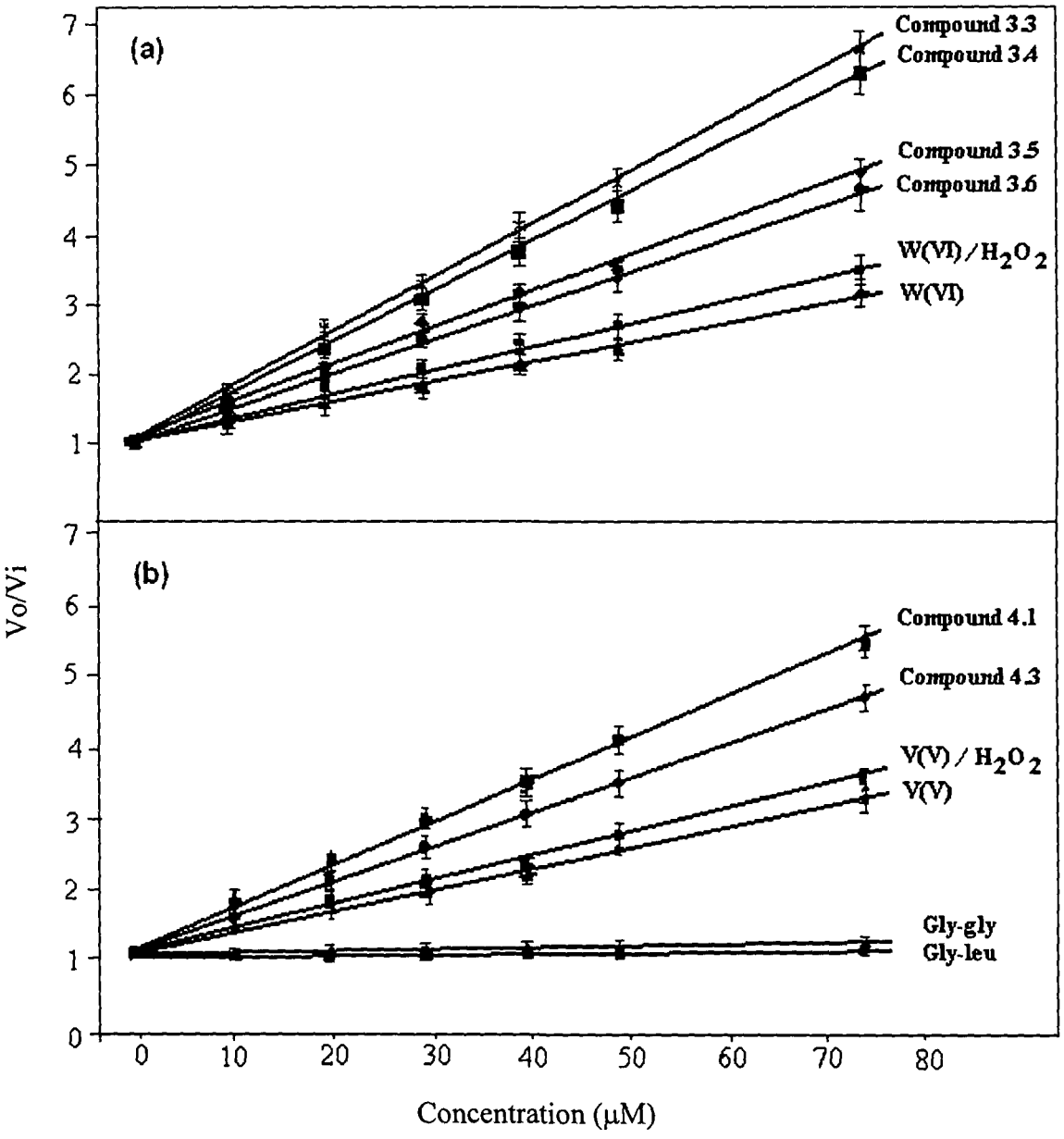


Fig. 5.7. V_0/V_i ratios for the inhibition of **(a)** pW compounds **3.3 – 3.6**,tungstate, W/H_2O_2 and **(b)** pV compounds **4.1** and **4.3,** gly-gly, gly-leu, vanadate, $V(V)/H_2O_2$ species in the alkaline phosphatase catalyzed hydrolysis of p-NPP. The assay of the reaction medium was similar to that described under *Fig. 5.6.*

IC_{50} values that among the species tested, the newly synthesized heteroligand pV and pW compounds are the most potent inhibitors of the enzyme. From the trend observed it is also apparent that the pV complexes exert greater inhibitory effect compared to the corresponding tungsten containing analogues. The values obtained for V(V)-H_2O_2 or W(VI)-H_2O_2 systems do not differ drastically from that of vanadate or tungstate, respectively. The effect of ancillary ligands viz., cystine, gly-gly or gly-leu on ALP activity is practically negligible under the assay conditions used and H_2O_2 as such had no observable effect.

5.4 DISCUSSION

Action of catalase on the newly synthesized pW and pV compounds is a slow process in contrast to that of its natural substrate, H_2O_2 which demonstrate that the compounds act as weak substrates to the enzyme. It is also notable that among the monomeric pV and pW compounds tested, pW compounds were found to exhibit greater resistance to catalase with the rate of degradation of the diperoxovanadate complexes **4.1-4.4** being comparable to those of the dimeric tetraperoxotungstate compounds (Table 5.1). Furthermore, the greater resistance of the heteroligand diperoxovanadate complexes compared to free DVP, as well as the observation that in each set of compounds, gly-leu containing complexes showed a slightly greater rate of degradation compared to the respective gly-gly containing analogue, make it apparent that the ability of the complexes to act as substrate to catalase is sensitive to the ligand environment.

We have been particularly interested to investigate the interaction of the synthesized complexes with glutathione (GSH) in view of the literature report[9] where it has been

demonstrated that the higher efficacy of the pertungstate and permolybdate as insulinomimetic agents is a consequence of their oxidizing activity relative to GSH. Glutathione (GSH), the tripeptide γ-L-glutamyl-L-cysteinylglycine is the major non protein thiol found in all living cells, which plays the role of cellular reducing agent and antioxidant[30] and is involved in detoxification process of exogenous materials. Detoxification of hydroperoxides is carried out by glutathione peroxidase, which utilizes reducing equivalents of GSH, resulting in the formation of oxidized glutathione (GSSG). It also plays a central role in vanadium metabolism[31].

The peroxovanadates, **4.1-4.4** oxidized stoichiometric amounts of GSH to GSSG. It was however, quite intriguing to note that, although the pW compounds **3.1-3.6** undergo degradation under the effect of catalase with complete loss of peroxide, the oxidation activity of the pW compounds tested irrespective of being monomeric or dimeric, was limited to *ca.* 50% of that expected on the basis of the peroxide content measured for these complexes. The result of the investigation is consistent with the proposal implicating formation of a monoperoxo-W (VI) intermediate, which is probably inactive in GSH oxidation. Formation of an inactive monoperoxo intermediate from a catalytically active diperoxo tungsten or molybdenum complex during oxygen-atom transfer reactions mediated by such complexes is not unprecedented[32,33,34]. However, the observation that only half of the peroxo groups present per molecule of a pW complex participate in oxidation, whereas pV stoichiometrically oxidizes GSH utilizing all of its co-ordinated water molecules defies explanation at this stage.

Alkaline phosphatase is a membrane-bound zinc metalloenzyme. The probable functions assigned to the enzyme include: phosphohydrolysis of organic phosphomonoesters

of low molecular mass; phosphotransferase activity and protein phosphatase activity. The maximum activity is shown at pH 8 or above. The reactions catalysed by the enzyme possibly involve an enzyme-phosphate intermediate.

Previous studies indicated that phosphatases are, in general, inhibited by oxyanions such as vanadate[10,21-23], molybdate and tungstate[5,8]. Such inhibition is attributed to the formation of pentaco-ordinated or hexaco-ordinated structures which are often described as phosphate analogues[5,7-10,21-23] . Crans *et al.* also reported that some six- or seven-coordinate vanadium or pV compounds inhibit phosphatases, with their inhibitor potency being sensitive to the structure, oxidation state and the nature of phosphoproteins[23]. Five coordinated compounds are however, documented to be more potent inhibitors than the 6 or 7 co-ordinated ones[23]. The most noteworthy feature emerging out of the present investigation is that although individually each of the tested species inhibited ALP activity to varying degrees, inhibitor potency of the synthetic pV and pW compounds tested despite having hepta-coordinated metal center in each of them, is much higher than that expected from equivalent concentrations of the corresponding metalloxide or metal peroxide formed in solution The above observations demonstrate that structural analogy with the transition state or phosphate mimicry may not be the factors due to which the title complexes exert inhibitory effect on protein phosphtases. That the inhibitor potency of the compounds are sensitive to the nature of co-ligand environment is apparent from our data, which shows complexes with gly-gly as ancillary ligand to be more potent as inhibitors than the corresponding gly-leu containing analogue. Further, among the dimeric pW compounds the following trend in inhibitor potency has been noted: $[W_2O_3(O_2)_4(cystine)]^{2-}$ > $[W_2O_3(O_2)_4(gly\text{-}gly)_2]^{2-}$ > $[W_2O_3(O_2)_4(gly\text{-}leu)_2]^{2-}$. However, the possibility of the observed

inhibition being caused by the amino acid or peptide co-ligand alone may be ruled out since the effect of individual ligand on ALP activity is practically negligible under the assay conditions used. Due to the complexity of the reactions and species involved in the present study, we refrain from drawing any conclusion regarding cause of the effect of title compounds on the phosphatase activity in absence of direct evidence. Nevertheless, on the basis of our observations made, in conjunction with the reports documenting the importance of redox properties of peroxo vanadium compounds in inhibition of protein phosphatases[21,23], the oxidizing ability of the synthesized pW and pV complexes may be expected to be one of the possible factors responsible for making the compounds effective inhibitors of the phosphatase. It has been shown earlier that redox reaction can occur between V(V) compounds and thiol group of the phosphatases thereby irreversibly modifying the protein[27]. Furthermore, peroxovanadate effectively inhibited the tyrosine phophatase by oxidizing the catalytic cysteine of the enzyme[35]. It is also tempting to suggest that higher inhibitor potency of pV **(4.1-4.4)** compounds is probably a consequence of their being more effective oxidants of GSH compared to pW.

In conclusion, the present experiments establish that the pW compounds(**3.1-3.6**), irrespective of being monomeric or dimeric and pV compounds (**4.1-4.4**), are potent inhibitors of ALP activity. It is also noteworthy that the title compounds are relatively resistant to degradation by the powerful enzyme catalase and the pW compounds utilise their peroxide groups only partially during interaction with GSH. These may be relevant in the cellular milieu where H_2O_2 has little chance to survive abundant catalase and glutathione peroxidase.

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CHAPTER 6

Dinuclear and mononuclear peroxotungsten(VI) complexes as oxidants in mild oxidative bromination*

6.1 INTRODUCTION

Bromination of organic substrates, particularly aromatics, has been attracting considerable contemporary interest[1-8] mainly due to the commercial importance of such compounds[9]. Manufacture of a range of chemicals, including antibacterial and antifungal drugs, agrochemicals, flame-retardants and dyes involves bromination[10]. Traditional bromination methods require the use of elemental bromine and solvents, which are environmentally hazardous[11].

Vanadium Bromoperoxidases (V-BPO), the enzyme involved in the biosynthesis of a variety of naturally occurring brominated products, catalyze bromination by using H_2O_2 and bromide salts instead of Br_2[12,13]. By itself, H_2O_2 is capable of oxidizing bromide in highly acidic medium (pH<3) but is ineffective in solution at pH>5.0. The enzyme functions explicitly in catalyzing rate determining bromide oxidation to generate an oxidized bromine species capable of transferring bromine atoms to acceptor molecules with electron rich π bonds[14]. The oxidized bromine intermediate is likely to be equivalent of hypobromous acid (HOBr), bromine (Br_2), tribromide (Br_3^-), or an enzyme-trapped bromonium ion[12,13] although, its exact speciation is still a matter of speculation. Taking

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cues from the knowledge of activity of V-BPO there have been continued efforts to develop alternative bromination protocols[13-19] and to generate environmentally benign catalytic systems for synthesis of brominated organics[8,15].

Studies on synthetic peroxovanadate complexes as functional and structural models of bromoperoxidase[13-19], have been immensely useful in helping to elucidate the details of mechanism of action of the enzyme and have provided diverse approaches to this area[13-21]. Contrary to natural V-BPO, which is most efficient at pH 5.5-7, several model complexes were found to be catalytically active in acid medium[13-22,23] which limits their utility as effective catalyst. It is worth mentioning in this context that a number of previously reported dinuclear heteroligand peroxovanadate compounds with a bridging μ-peroxo group,[24-26] could instantaneously oxidize bromide to a bromination competent intermediate in phosphate buffer at near neutral pH, also efficiently mediated bromination of organic substrates in aqueous-organic media. Besides vanadate and pV systems, oxidation of bromide by hydrogen peroxide has been reported to be catalyzed by tungstate(VI) systems in acidic medium[22]. A tungstate-exchanged layered double hydroxide has also been studied as a heterogeneous catalyst in oxidative bromination of olefines by H_2O_2 system[8,9]. However, examples of well-defined synthetic heteroligand peroxotungstate compounds displaying activity in oxidative bromination is scanty in literature[27] despite the number of reports dealing with the activity of pW compounds as stoichiometric or catalytic oxidants in organic oxidations[28,29,30].

Taking the above observations into account in the present work we focused on investigating the activity of newly synthesized pW compounds, **3.1-3.6** in peroxidative bromination. The objective of the present study was to find an oxidant of bromide with

good activity at physiological pH, an essential requirement of a biomimetic model. The existing reports confirming the role of oxo-bridged tetraperoxo as well as neutral diperoxo tungsten species in a variety of organic oxidation reactions[31], allowed us to foresee potential of the newly synthesized pW compounds as bromide oxidants. On the other hand, from our earlier experience with the reactivity of peroxovanadate[24], we did not expect the diperoxovanadate complexes, **4.1-4.4** to be effective in bromination at neutral pH.

Chapter **6** of the thesis presents an account of the reactivity of complexes, $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$ [A = Na (**3.1**) or K (**3.2**)], $Na_2[W_2O_3(O_2)_4(dipeptide)_2].3H_2O$ [dipeptide = glycyl-glycine (**3.3**) and glycyl-leucine, (**3.4**)] and $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$ [dipeptide = glycyl-glycine (**3.5**) or glycyl-leucine (**3.6**)] in oxidative bromination. The heteroligand diperoxovanadium(V) compounds were totally inactive as bromide oxidant under similar reaction conditions. as anticipated

6.2 EXPERIMENTAL SECTION

6.2.1 Measurements of bromination activity in solution

The method of de Boer et al.[32] of introducing four bromine atoms into the molecule of phenol red (ε^{433} mmol=19.7) to form bromophenol blue (ε^{592} mmol=67.4) was used to measure bromination activity. Phenol red acts as an efficient trap of active bromine species until it is exhausted, without influencing the rate of reaction. The

reaction mixture contained phosphate buffer (50 mM, pH 5.5), KBr (0.5 M) and phenol red (0.1 mM) kept at 30°C. The redox activity was tested by adding a measured amount of aliquot from solution of the synthesized compound (1 mM), and by monitoring the possible change in absorbance at 592 nm. The volume of the reaction mixture was kept at 25 ml. Aliquots were transferred to the spectrophotometer immediately after mixing.

6.2.2 Bromination of organic substrates and product analysis

In a representative procedure, organic substrate (0.5 mmol) was added to a solution of acetonitrile: water (1:1) (3 ml) containing KBr (1.5 mmol). A weighed amount of solid peroxotungstate complex (0.25 mmol of **3.3** or 0.5 mmol of **3.5**) was then added to the reaction mixture at room temperature under continuous stirring. The stirring was continued for *ca.*1 h. Reaction products as well as un reacted organic substrates were then extracted with diethyl ether and dried over anhydrous Na_2SO_4. Products were then separated by TLC and HPLC. ^{1}H-NMR spectroscopy and melting point determinations were made to interpret the products.

In order to isolate the tungsten species remaining after the completion of the reaction, the following method has been adopted. After extraction of the organic reaction product, the aqueous part of the reaction mixture was transferred to a 250 ml beaker. Keeping the solution in an ice bath, pre cooled acetone was added with constant stirring until a colorless pasty mass separated out. After allowing it to stand for 10 min in an ice bath, the supernatant liquid was decanted off and the residue was treated repeatedly with acetone under scratching until it became white microcrystalline solid. The product was

separated by centrifugation, washed with cold acetone and dried in vacuo over concentrated sulfuric acid.

6.3 RESULTS AND INTERPRETATION

6.3.1 Redox activity of the complexes in bromination reaction in solution

The bromination of phenol red to its tetra brominated product, bromophenol blue was used to measure the bromination activity of the complexes **3.1-3.6** in solution. Phenol red acts as an efficient trap of active bromine species and undergoes stoichiometric bromination reaction, which can be monitored conveniently using electronic spectroscopy (*Fig.6.1*).

Addition of freshly prepared aqueous solution of each of the compounds **3.1-3.6**, at concentrations indicated (Table 6.1), to the standard reaction of bromide in phosphate buffer in presence of phenol red resulted in gradual color change of the solution from yellow to blue. The spectrum recorded showed a peak at A_{592} characteristic of the product bromophenol blue and a decrease in absorbance of the peak at A_{433} due to loss of phenol red (*Fig. 6.1 & 6.2*). The data in Table 6.1 show that the dinuclear and mononuclear pW complexes, **3.1-3.5** possess bromination activity.

A similar reaction when carried out in absence of phenol red displayed a peak at 262 nm with a shoulder at 237 nm on addition of solutions of compounds (Fig. 6.3). Addition of phenol red to this solution resulted in the decrease in A_{262} nm and a peak at

Table 6.1 *Bromination of phenol red with peroxotungstate complexes* **3.1** and **3.3-3.6**

No.	Compounds	Conc(mM)	Rate of bromine transfer		Total bromine transfer (extrapolated to 1mM compound)
			ΔA_{592}/min	μM Br/min	mM Br/mM compound
1.	$Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ (**3.1**)	0.1	0.19	11.2	1.96
2.	$Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$ (**3.3**)	0.1	0.16	9.5	2.01
3.	$Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$ (**3.4**)	0.1	0.18	10.9	1.88
4.	$[WO(O_2)_2(glyl\text{-}gly)(H_2O)].3H_2O$ (**3.5**)	0.1	0.10	6.5	0.97
5.	$[WO(O_2)_2(glyl\text{-}leu)(H_2O)].3H_2O$ (**3.6**)	0.1	0.09	5.2	1.08

592 nm appeared indicating the formation of bromophenol blue. The 262 nm peak, therefore, represents a bromination competent oxidized species of bromide, probably an equilibrium mixture of BrOH, Br_2 and Br_3^- as proposed earlier[12].

Rates of bromination mediated by monomeric diperoxo compounds were recorded to be nearly half of the dinuclear tetraperoxo compounds under analogous reaction conditions. It was of significance to note that the bromination activity of each

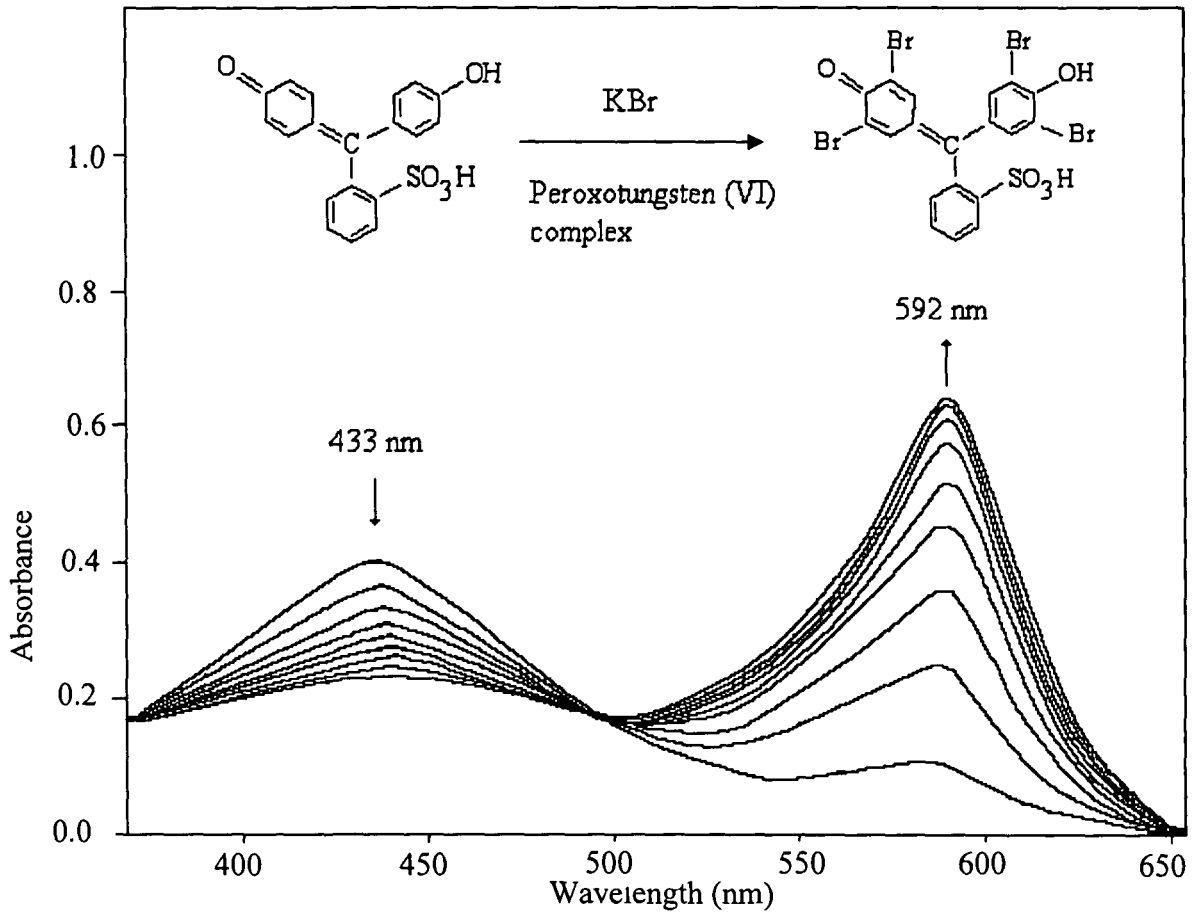


Fig. 6.1. Bromination activity with dinuclear peroxotungstate compound **3.3**. Spectral changes at 2 minutes interval after adding the compound solution to the reaction mixture. The reaction mixture contained phosphate buffer (0.05 M, pH 5.5), KBr (0.5 M), phenol red (0.1 mM) and compound **3.3** (0.05 mM).

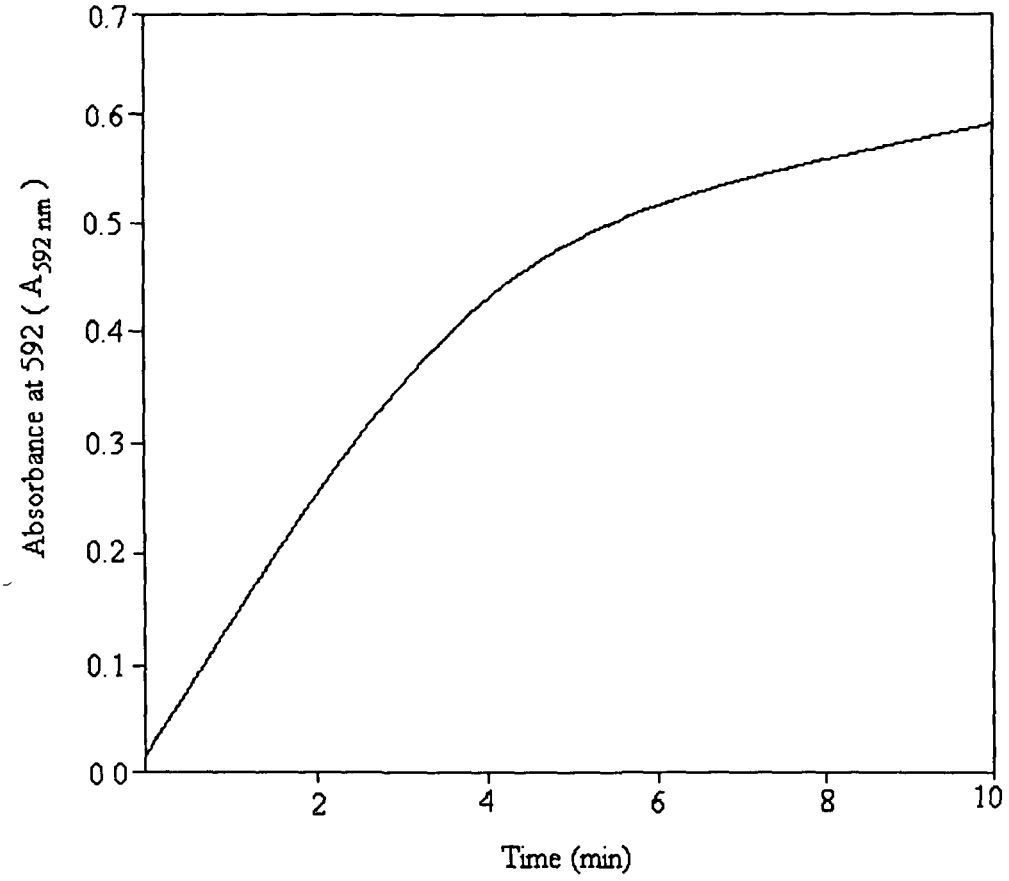


Fig. 6.2 The increase of absorbance at 592 nm indicating the rate of bromination with compound **3.3**. The reaction mixture contained phosphate buffer (0.05 M, pH 5.5), KBr (0.5 M), phenol red (0.1 mM) and compound **3.3** (0.05 mM).

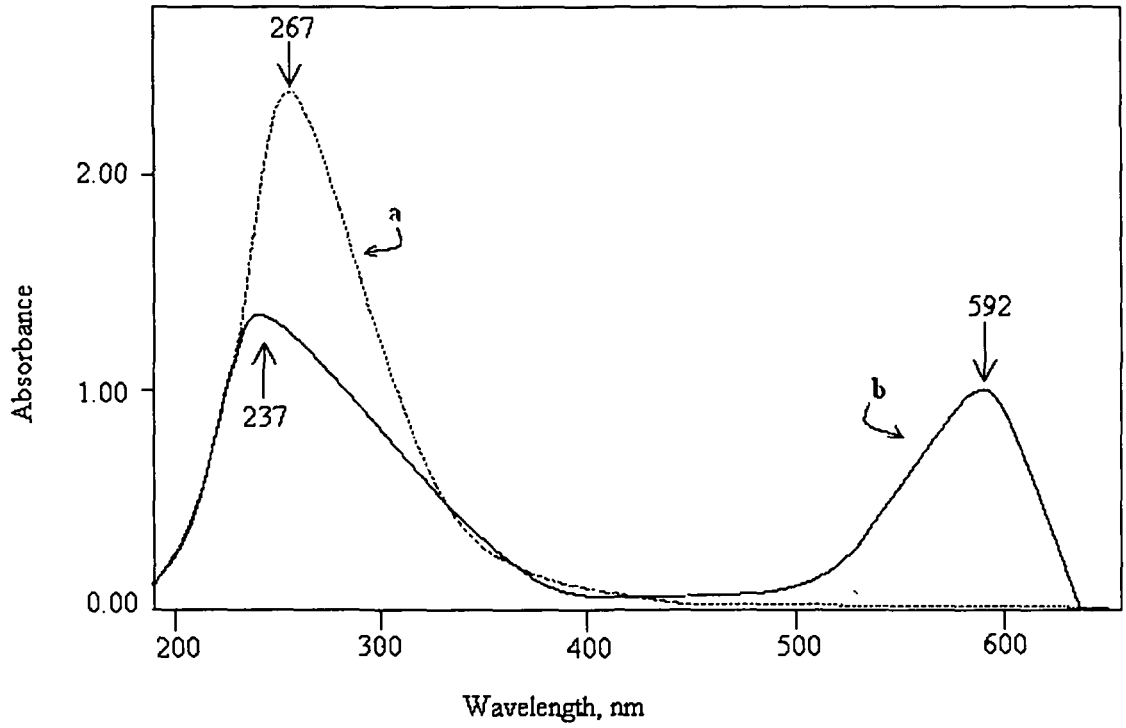


Fig. 6.3 Spectral changes following bromination of phenol red to bromophenol blue on addition of complex **3.3**. The reaction mixture contained phosphate buffer (0.05 M, pH 5.5) and KBr (0.5 M) (**a**) KBr + compound in absence of phenol red; (**b**) KBr + compound + phenol red (0.1 mM).

of the monomeric diperoxo and dimeric tetraperoxo complexes tested, like their oxidant activity with reduced GSH (*vide* Chapter 5*)*, were recorded to be limited to *ca.* 50% of that expected on the basis of the total number of peroxo groups present in the complexes (Table 6.1). Decrease or increase in concentrations of the compounds, substrate or KBr in the reaction solution had no effect on this feature. It is thus evident that in this case also, only one of the peroxo groups of a diperoxotungsten moiety of the monomeric complexes or two peroxo groups of tetraperoxo tungsten complex would be active in bromide oxidation.

6.3.2 Effect of H_2O_2 on peroxotungstate mediated bromination

The effect of H_2O_2 on the bromination reaction under standard assay condition was tested. No extra addition of H_2O_2 is required for the stoichiometric bromination reaction of the substrate by the pW compounds. While the initial addition of H_2O_2 (0.5 mM) to the reaction solution had no observable effect on the initial rate of bromination (*Fig.6.2*), it was quite intriguing to note a revival of the bromination activity on addition of H_2O_2 (0.5 mM), after bromination to a spent reaction mixture, which contained excess bromide and substrate. It is reasonable to assume that an inactive tungsten intermediate formed after completion of the bromination process probably combines with peroxide in presence of excess H_2O_2 to regenerate the respective active brominating species giving rise to a catalytic cycle. Exogenous hydrogen peroxide is therefore required to maintain the tungsten complexes in their active forms in order to obtain a catalytic cycle.

6.3.3 Effect of buffer

The bromination activity of the compounds was surprisingly, found to be maximum at pH 7.0 (*Fig 6.4*) unlike in the case of peroxovanadate catalyzed oxidative bromination where the rate was reported to increase monotonously with increasing acidity of the reaction medium[33]. We have obtained the data in Table 6.1 at pH 5.5 since the use of this method is limited to pH range near 5.0 in order to achieve conversion from phenol red (pKa 7.9) to bromophenol blue (pKa 4.0). Omission of phosphate buffer from the reaction medium did not alter the bromination activity of the complexes. This

indicated that the presence of phosphate was not essential for such activity of the peroxotungsten compounds.

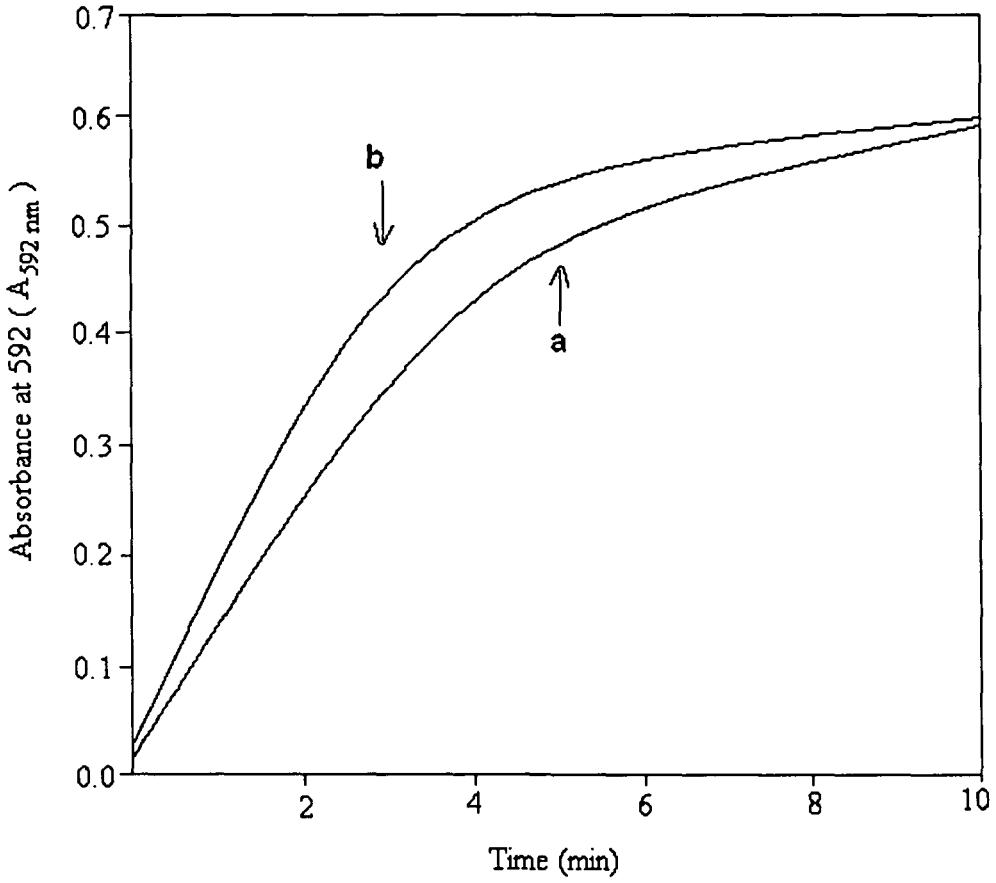


Fig. 6.4 The increase of absorbance at 592 nm indicating the rate of bromination with peroxotungsten compound **3.3** in phosphate buffer (0.05 M) of pH 5.5 (a) and pH 7.0 (b). The reaction mixture contained KBr (0.5 M), phenol red (0.1 mM) and compound **3.3** (0.05 mM).

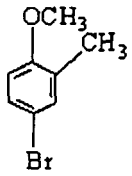
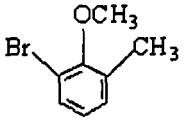
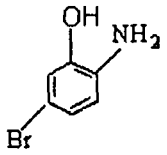
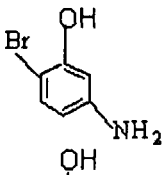
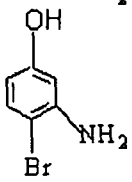
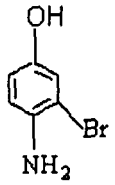
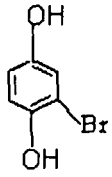
6.3.4 Substrate bromination in aqueous-organic media - evidence for electrophilic bromination

Efficacy of the pW complexes in mediating bromination of organic substrates in presence of bromide in aqueous-organic media has been explored by using dinuclear complexes **3.1** and **3.3**, and monomeric complex **3.5** as representatives. Bromination of several activated aromatics into their corresponding bromo-organics took place in moderate yields in presence of the dinuclear as well as mononuclear pW complexes **3.3** and **3.5** (Table 6.2) at ambient temperature. Reactions were carried out in the absence of buffer. The conditions of reactions such as reaction temperature substrate:oxidant stoichiometry, bromide concentration and type of solvent were optimized using the substrate p-nitroaniline as a representative. The solvent CH_3CN: H_2O (1:1) provided good yields of the products.

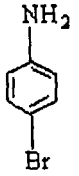
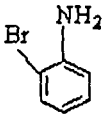
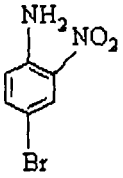
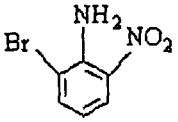
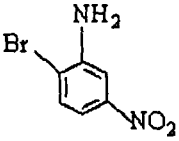
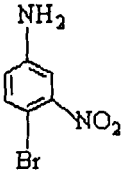
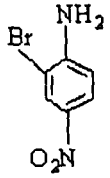
Preferential bromination at either ortho or para positions of the aromatic ring leading to mono substitution indicate an electrophilic bromination mechanism. Further mechanistic information about the reaction came from the study with the substrate 2-methoxytoluene, especially chosen for the purpose. That the brominating species was 'Br^+' and not a '$Br^\bullet$' in these reactions was further evident from the exclusive formation of ring substituted products, 3- or 5-bromo-2-methoxytoluene (*Fig. 6.5*). Bromination through radical reaction would have produced benzyl bromide[18]. The identity of all products was confirmed by melting point determination, comparison of their ^{1}H-NMR spectra (Table 6.2) with authentic sample and also by HPLC analysis.

Attempted bromination using pV complexes **4.1** and **4.3** under similar reaction condition did not yield brominated products as anticipated.

Table 6.2. *Bromination of organic substrates mediated by compound* **3.3**

Substrate	Product	Chemical Shift δ ppm	% Yield
2-Methoxytoluene		7.9(s, 1H), 7.4(d, J=6Hz, 1H), 6.85 (d, J=6Hz, 1H), 3.6(s, 3H, $-OCH_3$), 1.2(s, 3H, $-CH_3$)	58
		7.7-7.4(m, 3H), 3.64(s, 3H, $-OCH_3$), 1.1 (s, 3H, $-CH_3$)	34
o-Aminophenol		8.3 (s, 1H), 7.8(d, 1H, J=6Hz), 7.1(d, 1H, J=6Hz), 6.2-6.5 (br, 2H, $-NH_2$)	79
m-Aminophenol		7.8-7.25 (m, 3H), 5.3-5.0 (br, 2H, $-NH_2$)	60
		8.1-7.5 (m, 3H), 5.6-5.3 (br, 2H, $-NH_2$)	30
p-Aminophenol		8.3 (s, 1H), 7.4(d, 1H, J=6Hz), 6.9(d, 1H, J=6Hz), 6.2-5.9 (br, 2H, $-NH_2$)	76
Quinol		8.5 (s, 1H), 8.2-7.5 (m, 3H)	72

continued

Substrate	Product	Chemical Shift δ ppm	% Yield
Aniline		7.16 (d, 2H, J=7Hz), 6.5 (d, 2H, J=7Hz), 3.5 (s, 2H, $-NH_2$)	52
		7.5-6.36 (m, 4H), 4.0 (s, 2H, $-NH_2$)	35
o-Nitroaniline		8.3 (s, 1H), 7.45 (d, 1H, J=7Hz), 6.75 (d, 1H, J=7Hz), 6.35-6.05 (br, 2H, $-NH_2$)	61
		7.3-7.05 (m, 3H), 6.5 (s, 2H, $-NH_2$)	30
m-Nitroaniline		7.7-7.25 (m, 3H), 4.6-5.0 (br, 2H, $-NH_2$)	56
		7.5-6.7 (m, 3H), 4.5-4.8 (br, 2H, $-NH_2$)	30
p-Nitroaniline		8.25 (s, 1H), 7.9(d, 1H, J=6Hz), 6.64 (d, 1H, J=6Hz), 4.8-4.6 (br, 2H, $-NH_2$)	79

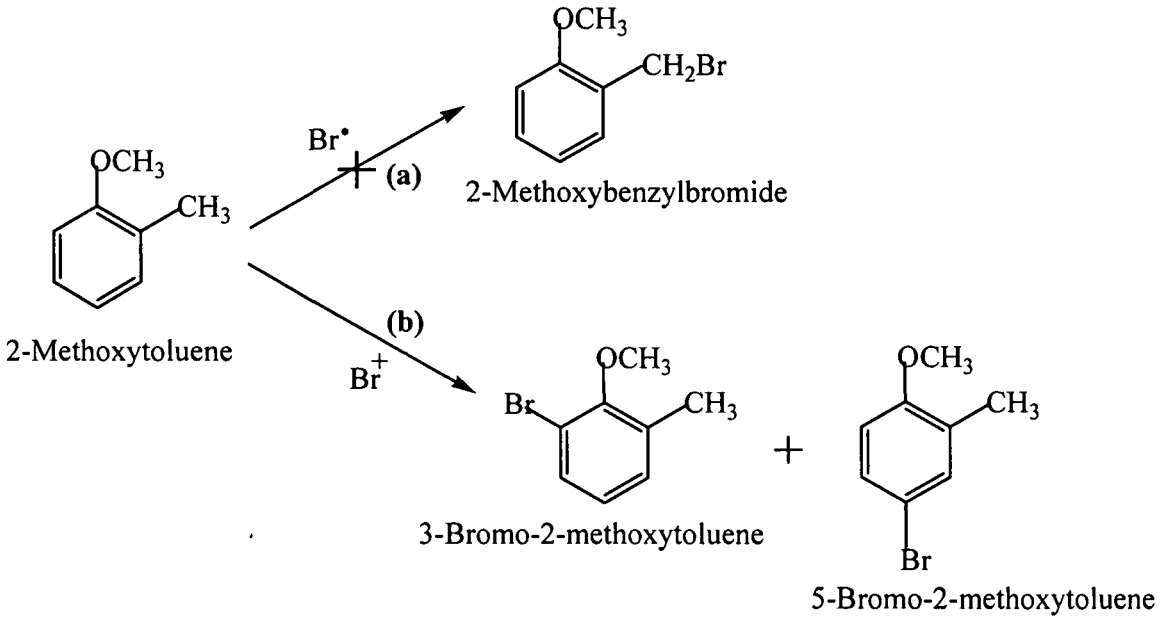


Fig. 6.5 Bromination reaction of 2-methoxytoluene. (a) Possible product of bromination through radical reaction, (b) electrophilic bromination involving 'Br^{+}' forms exclusively ring substituted products. Bromination reaction using dimeric or monomeric pW compound produces exclusively bromomethoxytoluene.

6.3.5 Identification of the inactive intermediate

In order to ascertain the nature of the inactive intermediate formed in solution and to rationalize the reaction sequence the product isolated from the aqueous extract of the reaction mixture, after completion of the reaction, was subjected to IR and elemental analysis. Bromination reaction mediated by the dimeric complex, **3.3** or its monomeric analogue, **3.5** were used separately as representative reaction for this purpose. IR

spectrum of the products isolated as above resembled closely the spectrum of the corresponding original starting complex in each case, showing the presence of peroxo group, bridging as well as terminal oxo groups and co-ordinated dipeptide. Elemental analysis results suggested the presence of one peroxo group per W(VI) indicating the formation of a monoperoxo tungsten species in each case. We could also isolate an analogous mono peroxotungsten species from a separate experiment involving complex **3.3** (0.5 mmol) and KBr (2.0 mM) under standard assay condition with phenol red omitted from the reaction medium. It is also evident from the findings that the dimeric oxo-bridged tungsten moiety of the compound **3.3**, remains intact throughout the course of the bromination process where two of its peroxo groups participate, leading to the formation of a dimeric peroxotungsten compound with two heteroligand monoperoxo moieties bonded through an oxo group.

6.4 DISCUSSION

Based on the afore mentioned observations a scheme of reactions, shown in *Fig. 6.6*, is proposed which satisfactorily describes principal features of our results. The dimeric compounds react with bromide to yield oxidized bromine species, proposed to be an equilibrium mixture of BrOH, Br_2 and Br_3^-, with concomitant formation of dinuclear intermediate which is likely to possess two monoperoxotungsten units with the oxo-bridge retained (reaction a). Similarly, a dioxo monoperoxo intermediate of the type, $[WO_2(O_2)L(H_2O)]$ (L = gly-gly or gly-leu) is expected to be formed from the

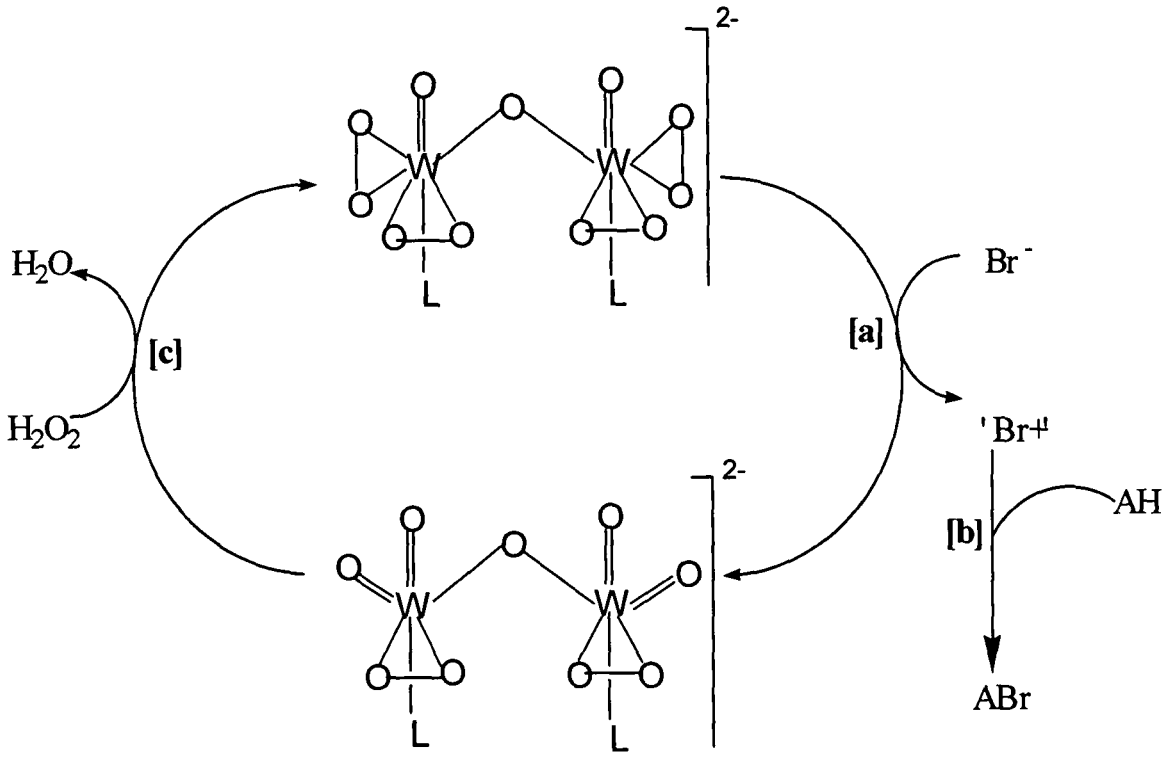


Fig. 6.6. Schematic representation of reactions occurring with dinuclear tetraperoxo–tungsten(VI) compounds (L = cystine, gly-gly or gly-leu). a) reaction of the complex with bromide to yield oxidized bromine and an intermediate postulated as a diperoxo-tungsten species having two monoperoxotungsten units with the oxo-bridge retained; b) transfer of bromine from the active species to acceptor AH; c) in presence of excess H_2O_2 the diperoxo intermediate combines with peroxide to regenerate the starting tetraperoxo tungstate complex giving rise to a catalytic cycle. No attempt is made to show the exact stoichiometry of the reaction.

corresponding monomeric oxidant **3.5** or **3.6**. Transfer of the bromine atom to the substrate AH from the bromination competent oxidised bromine intermediate takes place (reaction b). In the presence of excess H_2O_2 the inactive intermediate combines with peroxide to regenerate the starting tetraperoxo ditungstate complex (or the corresponding monomeric diperoxo analogue) giving rise to a catalytic cycle (reaction c). To us it appears that bromide would attack an edge-bound peroxo group in preference to a hepta co-ordinated tungsten centre as observed in some other redox processes involving peroxo compounds of W(VI) and Mo(VI)[27,34,35].

A monoperoxo Mo(VI) species has been implicated as an intermediate in the mechanisms proposed by Reynolds et al[27] as well as by Butler and co-workers for the Mo(VI) and W(VI) catalyzed bromide oxidations[22]. Support for such an inactive peroxotungsten intermediate also comes from our investigation on GSH oxidizing ability of pW compounds (*vide* Chapter 5) where only half of the peroxo groups out of total number of peroxides present per molecule of the complex were found to be involved in oxidation. These considerations fortify the proposed mechanism.

The observed reaction pattern is in accord with the earlier suggestions that for a peroxotungsten complex to be active in oxidation an oxo-diperoxo configuration may be a prerequisite[34]. This feature is however, in contrast to the findings that diperoxo-vanadate compounds with only η^2-peroxo groups in its co-ordination sphere were catalytically incompetent in bromide oxidation at neutral pH[24,33]. Dinuclear peroxovanadate compounds with a μ-peroxo group, on the other hand were highly active in bromination at physiological pH[23-26,33]. The proposed reaction pathway based on

our experiments and work of some other laboratories[23,33,36] conferred the status of a bromide oxidant, at physiological pH, on the VOOV group[23-26,33]. The mechanisms of bromide oxidation mediated by pW and pV compounds, tested by us, thus appear to be distinctly different. It is not completely clear however, as to why a η^2-peroxo group of diperoxo tungsten(VI) species is highly reactive in oxidative bromination while a diperoxovanadate species with similar edge-bound peroxo groups is ineffective in bromination at near neutral pH.

In summary, the present experiments confirm the reactivity of neutral monomeric, as well as dinuclear peroxotungsten compounds in producing a bromination competent intermediate at physiological pH. In view of the environmentally acceptable reaction condition of the bromination reaction mediated by these compounds which also includes redundancy of bromine or hydrobromic acid, the compounds may be considered as possible candidates of mimic of the function of the enzyme bromoperoxidase.

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CHAPTER 7

Summary and Conclusions

Interest in the chemistry of peroxovanadium complexes, as mentioned in Chapter **1**, has to a great extent been fueled by their potential catalytic[1-6], biochemical and therapeutic application[7-9]. In addition to increasing evidence of biological importance of tungsten[10,11], the efficiency of peroxo tungsten compounds as catalytic oxidizing and oxo-transfer agents in organic oxidations is notable[3,12-17].

Within the context of the present work, there are certain aspects of the chemistry of peroxotungstate and peroxovanadate that appeared to have received relatively less attention. Notably important among them is the absence of any reported existence of heteroligand oxo-bridged dinuclear pW complexes although, the anion $[W_2O_3(O_2)_4(H_2O)_2]^{2-}$ has been isolated and observed to be the active oxidant species in peroxotungsten catalyzed oxidations of organic substrates[17,18]. There is also a dearth of information on activity of discreet heteroligand peroxotungsten compounds in oxidative bromination[19], as well as their possible effect on inhibition or activation of enzyme functions. Moreover, despite the numerous reports dealing with synthesis and characterization of pV complexes[20-23]and solution studies on interaction of pV with biogenic species such as amino acids peptides and proteins[24-26], reports on synthetic pV containing such biogenic molecules as co-ligand is still very limited [27-29]. Strategic development of pV compounds with appropriate characteristics of relevance to biology remains a thriving area of research. Accordingly, we have embarked on a research programme designed to find suitable methods for the syntheses of well-defined and stable

peroxo heteroligand compounds of vanadium and tungsten and to derive information on some of their key properties of biological relevance, including their redox activity in biomimetic bromination.

In this Chapter, results of our studies on peroxo chemistry of vanadium and tungsten are summarized and some general conclusions are drawn from the observations made. Following are the notable points emerging out of the present investigation:

7.1 Synthesis of new heteroligand peroxo compounds of tungsten (VI) and vanadium(V)

(i) Synthesis of dinuclear oxo-bridged tetraperoxotungstate(VI) compounds can be achieved by stabilizing the species formed in a solution of tungstate and H_2O_2 at acidic pH, in presence of suitable amino acids or peptides serving as ancillary ligands, under appropriate reaction conditions. The ligands seem to stabilize the oxo-bridged complex ion by inter-ligand interaction, possibly hydrogen bonding.

Isolation of neutral monomeric products $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$ from similar reactions, conducted at relatively higher pH, underscore the importance of pH in achieving desired synthesis of hetero-ligand peroxotungsten compounds.

(ii) Reaction of V_2O_5 with H_2O_2 and the respective dipeptide ligand at pH *ca.*5 afforded the yellow diperoxovanadate(V) compounds $A[VO(O_2)_2(dipeptide)(H_2O)].H_2O$, (A = Na or K; dipeptide = gly-gly or gly-leu).

(iii) One of the notable common features between peptide coordinated dinuclear and mononuclear pW compounds **3.3-3.6** and pV compounds **4.1-4.4** is the occurrence of the ancillary ligands, gly-gly and gly-leu in their unidentate zwitterionic forms. Relatively low pH of the reaction medium maintained in the range of 2.5 -5.5 appears to be responsible for occurrence of the peptides as zwitterions and their preferential co-ordination through carboxylate group to W(VI) and V(V) , in each of the compounds.

7.2 Stability of the compounds in solution – action with catalase

(i) The newly synthesized pV and pW compounds are stable towards decomposition in solutions of a wide range of pH values, including acidic pH, for a reasonable period of time. In contrast to the highly unstable nature of the peptide containing dinuclear triperoxovanadate compounds, $[V_2O_2(O_2)_3(dipeptide)_3].H_2O$ reported earlier[27,28], the stability of the monomeric diperoxovanadate compounds **4.1- 4.4** in solution was found to be comparable to that of the tungsten containing analogues. The observation on enhanced stability of these pV compounds lends further credibility to our earlier suggestion that the monomeric diperoxovanadate complexes possessing exclusively chelated peroxo groups are more stable in solution as compared to the peroxo-bridged dimeric peroxovanadates, in similar co-ligand environment[27].

(ii) It is interesting to note that the synthesized pW and pV complexes serve as substrates, although poor ones, for catalase. This powerful enzyme leads to degradation of the compounds at rates 20-80 times slower compared to H_2O_2, the natural substrate of catalase.

7.3 Redox activity of the pW and pV complexes

7.3.1 Oxidation of GSH

The peroxovanadates, **4.1-4.4** oxidized reduced GSH to GSSG quantitatively. On the other hand, oxidant activity of the pW compounds tested irrespective of being monomeric or dimeric, was limited to *ca.* 50% of that expected on the basis of the peroxide content measured for these complexes.

7.3.2 Peroxotungsten compounds mediate oxidative bromination

(i) The dinuclear tetraperoxo as well as neutral monomeric diperoxo compounds **3.1** to **3.6** stoichiometrically oxidized bromide to a bromination competent intermediate in phosphate buffer at physiological pH, a reaction in which only half of the total number of peroxo groups of the complex species were found to be active. The bromination reaction can be made catalytic by the addition of exogenous H_2O_2.

(ii) The bromination activity of the compounds was observed to be maximum at pH 7.5, unlike in the case of peroxovanadate catalyzed oxidative bromination where the rate was reported to increase monotonously with increasing acidity of the reaction medium [30].

(iii) The peroxotungsten compounds are also efficient in mediating bromination of aromatic substrates in aqueous - organic media.

(iv) Peroxovanadium compounds **4.1-4.4** are totally inactive in bromination, which is in accord with the observation made earlier that DPV or compounds containing such moieties were catalytically incompetent in bromide oxidation at neutral pH[27,30]

The results of investigation on redox activity of the pW compounds are consistent with the proposal implicating formation of a monoperoxo-W(VI) intermediate, which is probably inactive in oxidation of GSH as well as bromide. However, there appears to be no plausible explanation as to why only half of the peroxide groups present per molecule of a pW complex should participate in oxidation whereas diperoxovanadate compounds stoichiometrically oxidized GSH by utilizing both of its coordinated peroxo groups.

7.4 Effect on alkaline phosphatase activity

Each of the pW and pV compounds exert strong inhibitory effect on alkaline phosphatase activity with a potency significantly higher compared to that of the corresponding free ligand, tungstate, peroxotungstate, or vanadate and peroxovanadate. Since the compounds possess 7 co-ordinated metal centre in each of them, the observed inhibitory effect of the complexes may be attributed to some stabilizing factors other than structural analogy with the transition state, or phosphate mimicry. Effect of individual ligands on ALP activity is practically negligible under the assay conditions used and H_2O_2 as such had no observable effect. It is reasonable to expect that redox properties of

the complexes which is also evident from its ability to oxidize GSH should be one of the factors responsible for making the compounds potent inhibitors of the phosphorylase.

7.5 Future prospects

The newly synthesized compounds represent a set of water-soluble peroxo derivatives, which contain molecules familiar to bioenvironment as heteroligands. A distinctive feature of these pW as well as pV compounds, which may be of clinical relevance, is their reasonably high stability towards decomposition in solution of a wide range of pH values, particularly at acidic pH. This may be significant in view of the observation made by Shisheva et al.[31] that peroxovanadate, when administered orally was ineffective in inducing normoglycemia in STZ-rats probably because it could not survive the strong acidity of the stomach. It is also notable that the degradation of the compounds under the effect of catalase is much slower compared to H_2O_2. Although the observed stability of these synthetic pV or pW complexes may not imply their stability *in vivo* after administration and uptake by cells, however, it fulfils one of the criteria for metal complexes to be useful as therapeutic agents and provide future scope for testing such properties.

We may also expect the compounds to be of biochemical interest in view of the properties such as their being efficient oxidants of GSH and their significant affinity as alkaline phosphatase inhibitor. In absence of direct evidence at this stage however we are, unable to discern definitive reasons for the effect of the title compounds on the phosphatase activity. Further investigation involving effect of other well-defined and

stable peroxo-metallates in diverse ligand environment which remain intact in solution, on the activity of the phosphatases combined with speciation analysis are likely to help in establishing structure-activity correlation of the compounds and in gaining an insight into the mechanism involved in such enzyme inhibitory processes. This aspect is currently being studied in our laboratory.

One of the most notable aspects of the present study is undoubtedly, the finding that the synthesized pW complexes besides being stoichiometric reagents for bromide oxidation in aqueous medium, also acted as catalyst for the same reaction when used in conjunction with H_2O_2 at neutral pH. The remarkable feature of the methodology is that the bromination reaction takes place at near neutral pH and no extra addition of acid or H_2O_2 is required for the stoichiometric bromination reaction of the substrate. These observations sustain hope that the information generated from the present investigation would find relevance in the context of designing bioinspired synthetic oxidants for organic bromination, which constitute an active area of current research[29-31].

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List of Publications

Papers published /accepted for publications

1. ***New oxo-bridged peroxotungsten complexes containing biogenic co-ligand as potent inhibitors of alkaline phosphatase activity.***
   Pankaj Hazarika, Diganta Kalita, Swapnalee Sarmah and Nashreen S. Islam*
   Mol. Cell. Biochem., 2006, **284**, 39.

2. ***New oxo-bridged dinuclear peroxotungsten(VI) complexes. Synthesis, stability and activity in bromoperoxidation.***
   Pankaj Hazarika, Diganta Kalita, Swapnalee Sarmah, Nashreen S. Islam*
   Polyhedron, 2006, **25**, 3501.

3. ***New peroxovanadium compounds containing biogenic co-ligands. Synthesis, stability and effect on alkaline phosphatase activity.***
   Pankaj Hazarika, Swapnalee Sarmah, Diganta Kalita, Nashreen S. Islam*
   Trans. Met. Chem., *2007* (in press)

4. ***Mononuclear and dinuclear peroxotungsten complexes with co-ordinated dipeptides as potent inhibitors of alkaline phosphatase activity.***
   Pankaj Hazarika, Diganta Kalita, Nashreen S. Islam*
   J. Enz. Inhib. Med. Chem., 2007 (in press)

Other publications

1. ***Peroxo-bridged divanadate as a selective bromide oxidant in bromoperoxidation.***
   Swapnalee Sarmah, Pankaj Hazarika, Nashreen S. Islam*, A. V. S. Rao, T. Ramasarma
   Mol. Cell. Biochem., 2002, 236, 95.

2. ***Reaction of diperoxovanadate with vanadyl sulphate in presence of EDTA as an access to dinuclear peroxovanadates(V).***
   Swapnalee Sarmah, Pankaj Hazarika, Nashreen S. Islam*
   Polyhedron, 2002, 21, 389.

3. ***Synthesis of new dinuclear and mononuclear peroxovanadium(V) complexes containing biogenic co-ligands: a comparative study of some of their properties.***
   Swapnalee Sarmah, Diganta Kalita, Pankaj Hazarika and Nashreen S Islam*
   Polyhedron, 2004, **23**, 1097.

4. ***Synthesis and characterization of novel catalase resistant monoperoxo divanadate(V) compounds.***
   Swapnalee Sarmah, Diganta Kalita, Pankaj Hazarika, Nashreen S Islam*
   Ind. J. Chem., 2005, **44A**, 2003.

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New oxo-bridged peroxotungsten complexes containing biogenic co-ligand as potent inhibitors of alkaline phosphatase activity

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Abstract

Novel dinuclear peroxo complexes of tungsten with coordinated cystine of the type $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$, A = Na (1) or K (2) have been synthesized from the reaction of A_2WO_4, cysteine and 30% H_2O_2 at pH 2.5. The synthesized compounds were characterized by elemental analysis, spectral and physico-chemical methods. The two W(VI) centres with side-on bound peroxo groups of the dinuclear complex species are bridged by an oxo group and a cystine ligand, formed from the oxidation of cysteine. Cystine occurring as zwitterion binds the metal centers of the complex ion through O(carboxylate) atoms leading to hepta co-ordination around each W(VI). The compounds exhibit high stability toward decomposition in solution of acidic as well as physiological pH and serve as weak substrates to catalase, undergoing degradation in presence of the enzyme at a rate much slower relative to H_2O_2. The compounds efficiently oxidized GSH to GSSG, a reaction in which only two of the peroxide groups of the complex species were found to participate. The compounds induce strong inhibitory effect on alkaline phosphatase activity with a potency higher than that of the free cystine, tungstate, or peroxotungstate. (Mol Cell Biochem **284:** 39–47, 2006)

Key words: ALP inhibitor, cystine containing peroxotungstate, dinuclear heteroligand peroxotungstate, GSH oxidant, substrate to catalase

Introduction

The discovery of tungstoenzymes in thermophilic bacteria and hyperthermophilic archea [1] is considered as one of the most exciting developments in contemporary bioinorganic chemistry [1, 2]. With this discovery status of tungsten has been raised to a biologically relevant element. The possibility of the existence of other organisms that might utilize this element is yet to be explored [1]. Compounds of tungsten such as polyoxotungstates, particularly silicotungstates have been reported to show antiviral activity [3, 4] and were found to be potent inhibitors of HIV reverse transcriptase and RNA-dependant DNA polymerase [4]. These fascinating findings and the observations that tungstates and peroxotungstates (pW) present in a solution of W-H_2O_2, like vanadate and peroxovanadates (pV), were capable of inhibiting the hydrolysis of phosphoproteins [5–9] and stimulate most of the insulin bioeffects in rat adipocytes [6] led to an upsurge in interest in the biochemistry of the metal and its compounds.

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The exact mechanism by which peroxotungstates or peroxovanadates mimic the action of insulin or inhibit enzyme function is yet to be fully understood [6, 10–12]. However, correlation has been established between abilities of vanadate and pV to inhibit protein phosphatases and their insulin enhancing effect and in vivo insulin mimetic activities [10–13]. Shechter and co-workers observed [6] that the higher efficacy of the pertungstates and permolybdates as insulinomimetic agents originated from their oxidizing activity relative to GSH.

In order to gain an insight into the role of vanadium in bio-processes a variety of synthetic peroxo vanadium compounds with different ancillary ligands have been synthesized and studied as biomimetic models [13–16]. Moreover, such compounds have been tested and were found to have insulin like activities [13–16] as well as significant affinity as phosphatase inhibitors [10–12]. However, most of these compounds are hydrolytically unstable which limits their utility as therapeutic agents [12, 13]. On the other hand, despite the observation that peroxotungstates, formed in a solution of W-H_2O_2 were stable in solution of a wide range of pH values [6], there appears to be no information available pertaining to studies involving the effect of well-defined synthetic heteroligand peroxotungsten compounds on activity of different enzymes including phosphatases [2]. The importance of enzyme inhibition as a mode of action for inorganic drugs is being recognized in recent years and is an area needing exploration [3].

Chemistry of tungsten peroxo complexes have been receiving continued importance mainly due to their well established role as an important class of stoichiometric or catalytic oxidizing and oxo-transfer agent in a variety of organic oxidations [17–20]. A large number of heteroligand peroxo complexes of tungsten have been synthesized in recent years [20–25], however, there appears to be a paucity of information on synthesis of complexes with $[W_2O_3(O_2)_4]^{2-}$ moiety stabilized by a heteroligand other than H_2O [20–25]. The dimeric species have been known to exist in a solution of tungstate and excess peroxide at pH $\leq$ 5 and are believed to be the species responsible for tungstate catalysed oxidations by H_2O_2 [20–22]. Moreover, reports on synthetic peroxo-W(VI) complexes with co-ordinated amino acids are very few [20, 23]. Amino acids are probably the primary ligands to interact with the metal in biological system. A better understanding of the complexation behavior of tungsten with such ligands is therefore of vital interest. In view of the importance of cysteine and cystine in biological redox processes we were particularly interested to study the reaction of W-H_2O_2 with cysteine and expected that a dinuclear tungsten system with a S-containing ligand in its co-ordination sphere would constitute an interesting combination.

The above observations prompted us to direct our efforts in establishing viable synthetic routes to newer dinuclear oxo-bridged heteroligand peroxotungsten compounds with appropriate characteristics of solubility and stability and to study some of their biochemically relevant properties.

In this paper, the synthesis and physico-chemical characterisation of novel dimeric peroxotungsten compounds of the type, $A_2[W_2O_3(O_2)_4(\text{cystine})].4H_2O$ (A = Na or K) with the distinctive feature of having cystine as a bridging co-ligand in addition to a μ-oxo group, are presented. Results of investigation on their stability towards decomposition in solution and interaction with catalase and GSH are also described herein. The compounds were screened for ALP inhibition which revealed their strong affinity as inhibitor of the activity of the enzyme.

Materials and methods

Chemicals and solutions

The sources of chemicals are given below: Catalase, alkaline phosphatase from rabbit intestine, pNPP (Sigma-Aldrich Chemical Company, New Delhi); Sodium and potassium tungstates, cystine, cysteine (CDH, New Delhi, India); Hydrogen Peroxide (30%) (Ranbaxy, New Delhi, India); DTNB, GSH (Himedia laboratories, Mumbai, India); glycine, potassium dihydrogen phosphates, sodium and potasssium hydroxides, magnassium chloride (SD Fine Chemicals, Mumbai, India). Solutions were made fresh before the experiments in distilled water, doubly distilled in a quartz apparatus after initially passing through milli RO water purification system.

Synthesis of peroxotungstate complexes,

$$A_2[W_2O_3(O_2)_4(\text{cystine})].4H_2O(A = \text{Na or K})$$

In a typical reaction A_2WO_4 (0.5 g, 1.5 mM) was added to a solution of cysteine (0.24 g, 1.5 mM) in 5 ml of water. Keeping this mixture in an ice bath 5 ml of 30% H_2O_2 (44.1 mM) was added gradually with constant stirring until all solid dissolved and a clear colourless solution was obtained. The pH of the solution was recorded to be 2.5. No attempt was made to adjust the pH of the reaction solution. On adding pre-cooled acetone (ca. 50 ml) to the above solution under continuous stirring, a white pasty mass separated out. After allowing it to stand for about 10 min in the ice bath, the supernatant liquid was decanted and the residue was treated repeatedly with acetone under scratching until it became a microcrystalline solid. The product was separated by centrifugation, washed with cold acetone and dried in vacuo over concentrated sulfuric acid. In the solid state these complexes were found to be stable for several weeks stored dry at <20 °C but tended to be hygroscopic at ambient conditions and decomposed in a few days.

Anal. Calc. for $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$: Na, 4.91; W, 39.31; O_2^{2-}, 13.67; C, 7.69; N, 2.99; H, 1.28. Found: Na, 4.88; W, 39.26; O_2^{2-}, 13.63; C, 7.62; N, 2.90; H, 1.34. Yield: approximately 52%.

Anal. Calc. for $K_2[W_2O_3(O_2)_4(cystine)].4H_2O$: K, 8.05; W, 38.01; O_2^{2-}, 13.22; C, 7.43; N, 2.89; H, 1.24. Found: K, 7.90; W, 38.20; O_2^{2-}, 13.19; C, 7.36; N, 2.82; H, 1.32. Yield: approximately 54%.

Elemental analysis

The compounds were analysed for C,H,N at the Regional Sophisticated Instrumentation Centre, North Eastern Hill University, Shillong, India. The total peroxide content was determined by adding a weighed amount of the compound to a cold solution of 1.5% boric acid (w/v) in 0.7 M sulfuric acid (100 ml) and titration with standard Cerium(IV) solution. The values are given as % by weight of the compounds from which the ratios of W: peroxide are derived [26]. Tungsten was determined gravimetrically as $BaWO_4$ [27].

Physical and spectroscopic measurements

The IR spectra were recorded with samples as KBr pellets in a Nicolet model 410 FTIR spectrophotometer. The spectra were recorded at ambient temperature by making pressed pellets of the compounds. Specroscopic determinations of the initial rate of ALP catalysed hydrolysis of pNPP were carried out in a Cary model Bio 100 spectrophotometer, equiped with a peltier controlled constant temperature cell. The absorbance values were denoted as e.g. A_{405} at the wavelength indicated. Magnetic susceptibilities of the complexes were measured by the Gouy Method, using Hg[Co(NCS)] as the calibrant. Molar conductance measurements were made at ambient temperature using Systronics Conductivity Meter 306.

Stability of complexes in solution

Stability of the compounds in distilled water at pH 3.5, which is the natural pH of the compounds in solution, was studied by estimating the peroxide content in aliquots drawn from a solution of the compound **1** or **2** (0.1 mM) at different time intervals (Fig. 2) by the method described above. As a measure of stability of the compounds in solution molar conductances of the compounds at ambient temperature were recorded at 30 min gap for a period of 5 h. The values ($(263\ \Omega^{-1}cm^2mol^{-1}$ for **1** and $255\ \Omega^{-1}cm^2mol^{-1}$ for **2**) obtained at the beginning of the experiment remained unchanged during the period under investigation. Stability of the compounds at pH 7.0 was measured by titrating the peroxide content in compound solution (0.1 mM) in phosphate buffer (50 mM, pH 7.0). Stability of the compounds at pH 8 was similarly measured.

Eeffect of catalase on the complexes

The effect of catalase on complexes was studied by estimating the peroxide content of the compounds in a solution containing catalase at specified time intervals (Fig. 2). The test solution contained phosphate buffer (50 mM, pH 7.0) and catalase (40 μg/ml). The volume of the reaction solution was kept at 25 ml. The solution was incubated at 30 °C. The compound was then added to the test solution and aliquots of 5 ml were pipetted out and titrated for peroxide content after stopping the reaction by adding it to cold sulfuric acid (0.7 M, 100 ml) at time 5, 10, 15, 20, 25 and 40 min of starting the reaction. Three concentrations of peroxotungstate compound 1 (0.05, 0.1, 0.2 mM) were tested.

Measurement of redox activity in solution

To a reaction mixture containing GSH (80 μM) and phosphate buffer (50 mM, pH 7.0) a measured amount of aliquot from solution of the synthesized compound (1 mM) was added. Following incubation of 10 min, DTNB (160 μM) was added to the solution and the change in absorbance at 412 nm was determined in order to measure the GSH remaining in solution by the method of Ellman [28] using molar extinction of $\varepsilon_{412} = 13{,}600$. Measurements were done by using different concentrations of the compounds, 5.0, 10.0, 20 μM, in triplicate under same assay conditions.

Measurement of alkaline phosphatase activity

Phosphatase activity was assayed spectrophotometrically by using *p*-nitrophenyl phosphate (*p*-NPP) as a substrate. The continuous production of *p*-nitrophenol (*p*-NP) was determined at 30 °C by measuring absorbance at 405 nm in a reaction mixture containing ALP from rabbit intestine (3.3 μg protein/ml), *p*-NPP(1 mM) in incubation buffer (25 mM glycine+2mM $MgCl_2$, pH 10.0). The initial reaction rates were obtained by starting the reaction by adding ALP to the reaction solution which was pre-incubated for 5 min. The initial reaction rate of *p*-NPP hydrolysis in the absence of the inhibitors, V_0 was determined which was used as control. The effects of peroxotungstate compound **1** and other inhibitors were assessed by adding different concentrations (10–100 μM) of each species in the ALP assay. The V_i was obtained as the rate of *p*-NPP hydrolysis in the presence of

Table 1. The structurally significant IR bands of $A_2[W_2O_3(O_2)_4(cystine)].H_2O$ (A= Na or K)

No.	Compound	IR bands					
		$\nu(W{=}O)$	$\nu(O{-}O)$	$\nu(W_2O)$	$\nu_{as}(W{-}O_2)$	$\nu_s(W{-}O_2)$	$\nu_{sym}(W_2O)$
1	$Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$	962	878	744	605	520	410
2.	$K_2[W_2O_3(O_2)_4(cystine)].4H_2O$	957	875	744	605	518	415

variable concentrations of inhibitors under similar experimental conditions. The V_0/V_i ratios were calculated from these values. The concentrations tested for the compounds **1** and **2** and each of the other inhibitors were 10, 20, 30, 40, 50, 70 and 100 μM. The IC_{50} values were graphically determined as the half-maximal inhibitory concentration of the inhibitor species giving 50% inhibition. All the assays were performed in triplicate. The data in figures are presented as the means ± SE from three separate experiments.

Results

Synthesis and characterization

The methodology for the successful synthesis of the white microcrystalline compounds, $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$ was based on the reaction of A_2WO_4 with 30% H_2O_2 and $CysH_2$ in an aqueous acidic medium. Since one of our primary aims was to isolate complex with a $[WO(O_2)_2]_2O$ moiety which has been reported to exist in an acidic solution [21, 22] the reaction was strategically carried out at a pH 2.5, the natural pH of the reaction solution. The factors such as sequence of addition of the reactants as well as maintenance of required reaction time and temperature at $\leq$4 °C were found to be equally important for achieving the desired syntheses. Our attempts to synthesize similar dinuclear compounds in the presence of ligands such as glutamic acid, asparatic acid and thioglycolic acid yielded monomeric heteroligand peroxotungstate products in each case.

The title compounds were diamagnetic in nature as was evident from the magnetic susceptibility measurement in conformity with the presence of tungsten(VI) in each of them.

Valuable information regarding composition of the compounds was obtained from the elemental analysis data. A ratio of 1:2 was obtained for W: peroxide, while W: cystine ratio was ascertained to be 2:1 in each of the complexes suggesting a dimeric nature of the complex species. The elemental analysis results and molar conductance values obtained from measurement at ambient temperature (263 $\Omega^{-1}cm^2mol^{-1}$ for **1** and 255 $\Omega^{-1}cm^2mol^{-1}$ for **2**) were in complete agreement with the formulation of the complex species as $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$.

The IR spectra of the title compounds displayed a sufficiently well resolved spectral pattern main features of which are summarized in Table 1. The strong absorption at ca. 960 cm^{-1} was consistent with the presence of a terminally bonded W=O group in the complex [21, 22]. For the binuclear W—O—W unit, the antisymmetric and symmetric stretchings were expected in the 750 and 500 cm^{-1} region [21, 22], respectively. In the spectra of the complex, a prominent band at c. 748 cm^{-1} and another weak intensity band appearing at c. 420 cm^{-1} attributable to $\nu_{asym}(W_2O)$ and $\nu_{sym}(W_2O)$ modes gave clear indication of the presence of a bridging oxo group in each of them. Consequent upon the presence of side-on bound peroxo ligand, the expected $\nu(O{-}O)$ and ν_2 and ν_3 modes which involve metal-oxygen stretches of $W{-}O_2$ vibrations have been observed at c. 870, c. 610 and c. 520 cm^{-1}, respectively.

The existence of coordinated cystine in the complexes **1** and **2** was evident from their IR spectra which showed characteristic differences between the spectral pattern originating from the complexes and those of free cystine and cysteine [29]. The absorption attributable to $\nu_{as}(COO)$ of coordinated carboxylate group appeared at c. 1640 cm^{-1} in the spectra of the complexes. The symmetric stretching vibration of the carboxylate group of unco-ordinated ligand occur at 1430 cm^{-1}. The spectra of the compounds displayed a medium intensity band at ca. 1392 cm^{-1} which may be assigned to $\nu_s(COO)$. The shifting of this band to lower frequency with the difference of $\nu_{as} - \nu_s \approx 250\ cm^{-1}$ is characteristic of monodentate coordination of the carboxylate group via the O(carboxylate) atom [30]. Significant is the absence of S-H stretching (at c. 2565 cm^{-1}) in the spectra of the complexes, thereby implying either oxidation of the sulfhydryl group to its disulfide form or its deprotonation and subsequent co-ordination [29]. However, since no band attributable to ν(W-S) has been observed at c.390 cm^{-1} region [29], participation of S atom in co-ordination has been ruled out. On the other hand, the spectra of the complexes exhibited an intense band at 538 cm^{-1} characteristic of —S—S— moiety[29], thereby causing us to infer that the amino acid is present in the complex in its disulfide form i.e. as cystine and not cysteine. The bands appearing in the 3200–3000 cm^{-1} region and at c.1500 cm^{-1} were assigned to the N—H stretching and N^+H_3 symmetric deformation modes of the N^+H_3 group, respectively. The rocking modes of N^+H_3 occurred at c.1190 and 1049 cm^{-1}. It was

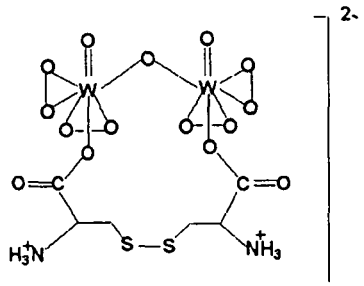

Fig. 1. A representation of proposed structure of the dinuclear peroxotungstate complex anion $[W_2O_3(O_2)_4(cystine)]^{2-}$.

thus evident that N atom do not participate in co-ordination in the title compounds. Presence of lattice water in the complexes **1** and **2** was indicated by the strong ν(OH) absorptions displayed at 3500–3400 cm^{-1}. However, the bending mode of water could not be assigned with certainty as it occurred in the carbonyl frequency region. The IR spectral data thus suggest that in the complexes **1** and **2**, cystine occurring as zwitterion binds the W(VI) centres through O(carboxylate) atoms.

The above results are consistent with a structure of the complex species shown in Fig. 1. In the dinuclear oxo-bridged complex ion the ancillary ligand acts as a bridge between the two peroxotungstate moieties thereby completing hepta coordination around each of the tungsten(VI) centres.

Stability of the complexes in solution – action with catalase and glutathione

The stability of the compounds in an aqueous solution of pH 3.5, which is the pH attained by the solution on dissolution of the compounds, has been studied by estimating the peroxide content and molar conductances at different time intervals for any possible change. The investigations revealed that their peroxide content and molar conductance values remained unaltered for over a period of 5 h and oxygen was not released on dissolution of the complexes in water. We further tested their stability at pH 7.0. Fig. 2 demonstrates that the compounds are highly stable at pH 7.0 as well as at pH 3.5. The compounds were also found to be stable at pH 8 (data not shown).

Keeping in view our goal of studying some of the biochemically relevant properties of the complexes, we considered it important to examine the effect of the enzyme catalase on the title compounds and their oxidizing ability with respect to reduced glutathione (GSH). Addition of catalase, the ubiquitous enzyme which catalyze the breakdown of H_2O_2 formed during oxidative processes in the intercellular peroxisomes, to phosphate buffered solution of H_2O_2 released

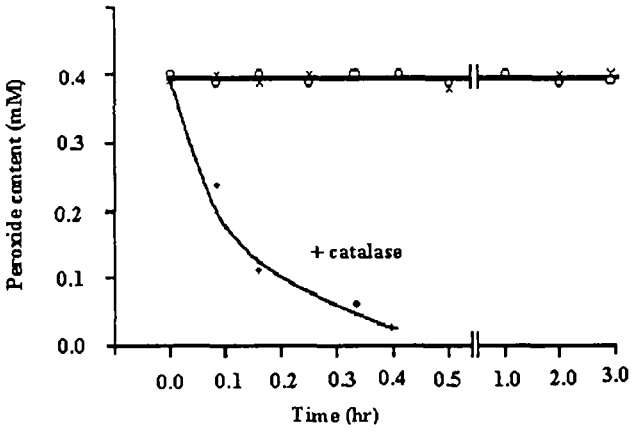

Fig. 2. Stability of complex 1 at different pH values, effect of catalase. □: compound solution in distilled water (0.1 mM), pH of the solution 3.5. ×: Solution of complex 1 in phosphate buffer (pH 7.0). ♦: effect of catalase. The test solution contained phosphate buffer (50 mM, pH 7.0) and the catalase (40 μg/ml) which was incubated at 30 °C for 5 min. Compound 1 (0.1 mM) was then added to the reaction solution and aliquots were drawn at indicated time points and loss in peroxide content was determined.

a half-equivalent (molecular basis) of oxygen, as expected from disproportionation reaction. On incubation with catalase, the compounds **1** and **2** were found to be degraded with the loss of peroxide at the rate c. 20 μM/min from a solution of 0.1 mM (Fig. 2) which contained c. 0.39 mM of peroxide. Tested at three concentrations of peroxotungstate compound 1, 0.05, 0.1, 0.2 mM, total peroxide loss was recorded to be 0.18, 0.37, 0.76 mM, respectively thereby indicating a ratio of 1:4 for peroxide: peroxotungstate complex **1**. Based on available report on other peroxometallate [31], it is reasonable to assume that the loss of peroxide from the complexes under the effect of catalase is accompanied by release of O_2. For the title compounds 2.0 O_2 per mole of the compound is expected to be released due to the presence of 4 peroxo groups per molecule. The total amounts of peroxide lost in the present case would then correspond to the release of c. 2.0 O_2 per mole of the compound. Under the effect of catalase the rate of degradation of H_2O_2 with the release of oxygen was reported to be 430 μM/min [31] from a solution of 0.1 mM concentration which is several fold higher than the rate of degradation observed for peroxo tungstate compounds under similar reaction conditions. These results suggest that the complex species acts as substrate for catalase, albeit a week one.

Oxidizing capacity of permetalloxides with respect to GSH, were tested by the method of Ellman [28]. Amounts of GSH oxidized at three different concentrations of the title compound **1**, 5, 10, 20 μM were recorded to be 20, 37, 78 μM, respectively. The compounds thus oxidized 4 moles of GSH to GSSG per mole of the compound. In view of the

presence of 4 peroxo groups in the complex, eight moles of GSH should have been oxidized per mole of the compound if all the four peroxo groups were active in oxidation. The stoichiometry of 4:1 for GSH oxidized: peroxotungstate compound was consistently obtained indicating that two of the four peroxo groups would probably be retained on each of the molecule of the compound. The absence of free —SH groups in the complex was ascertained by testing the solution with DTNB at the beginning of the experiment.

Effect on alkaline phosphatase activity

Using rabbit intestine alkaline phophatase, the established enzyme assay system and *p*-NPP as substrate, the effect of the newly synthesized complexes upon ALP activity was systematically investigated and compared with the effect induced separately by the free ligand, tungstate and peroxo tungstate generated in solution. Presented in Fig. 3 are the dose dependent effects of the complex 1 compared with the free ligand, W(VI) and W(VI)-H_2O_2 system, respectively. To quantify the inhibitory potential of the molecules, we determined the half-maximal inhibitory concentration (IC_{50}) for each inhibitor which gave rise to a 50% suppression of the original enzyme activity (Table 2).

We have also determined the enzymatic rate ratios of V_0/V_i where V_0 is the uninhibited rate and V_i is the rate of the enzymatic reaction inhibited by the complexes and other species.

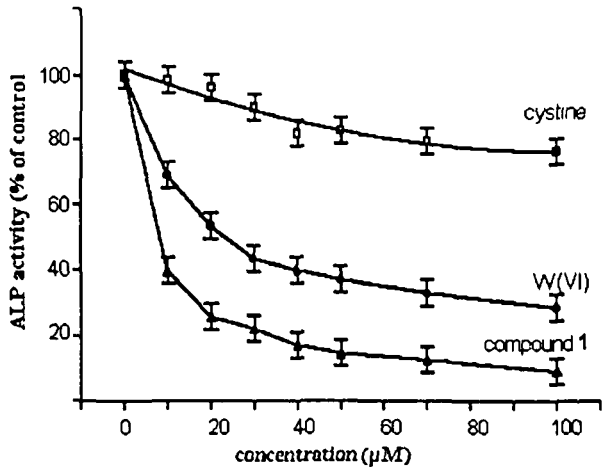

Fig. 3. Effect of compound 1, W(VI), cystine and W(VI)/H_2O_2 species on activity of ALP from rabbit intestine. The ALP catalyzed rates of hydrolysis of *p*-NPP at pH 10.0 were determined at 30 °C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/ml), *p*-NPP(1 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) in the absence or presence of stated concentrations of the inhibitors. Effects of the additions are represented as the percent values (rounded to integers) of control (Δp-NP = 0.96 μM/min). The data are presented as the means ± SE from three separate experiments.

Table 2. Half-maximal inhibitory concentration (IC_{50}) values of the compounds 1 and 2 and other inhibitors against ALP

Inhibitor		IC_{50}(μM)
1.	$Na_2[W_2O_3(O_2)_4$(cystine)].$4H_2O$	8.2
2.	$K_2[W_2O_3(O_2)_4$(cystine)].$4H_2O$	8.0
3.	Cystine	–
4.	Tungstate	28.2
5.	Tungstate/H_2O_2	27.7

Note. The ALP catalyzed rates of hydrolysis of *p*-NPP at pH 10.0 were determined at 30 °C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/ml), *p*-NPP(1 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) in the presence of stated concentrations of the inhibitors (Fig. 3).

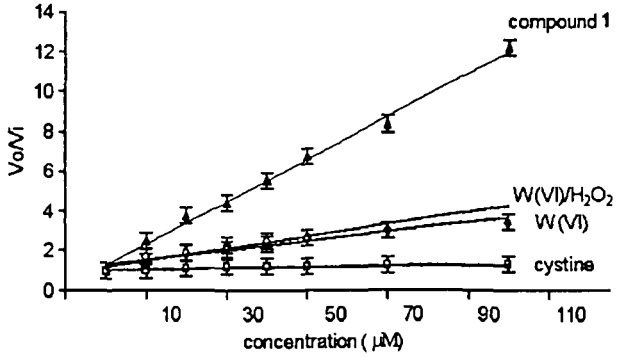

Fig. 4. V_0/V_i ratios for the inhibition of compound 1, W(VI), cystine and W(VI)/H_2O_2 species in the alkaline phosphatase catalyzed hydrolysis of *p*-NPP. The reaction mixture contained glycine buffer (25 mM glycine +2 mM $MgCl_2$, pH 10.0) and *p*-NPP(1 mM). The reaction was started by adding ALP (3.3 μg/ml) to the reaction solution which was pre-incubated for 5 min and the rate of hydrolysis in the absence of the inhibitors, V_0, was obtained. The inhibited rates of hydrolysis, V_i, were determined as above and in the presence of stated concentrations of inhibitors. The values are expressed as means ± SE from three separate experiments.

The V_0/V_i ratio was found to be directly proportional to the different compound concentrations. The results obtained can be seen in Fig. 4. From the Vo/V_i relationships and IC_{50} values it is evident that each of the tested species behaved as active inhibitor of ALP activity with the following order of potency compound 1 > W(VI)/H_2O_2 ≈ W(VI) > cystine. The values obtained for pW do not differ drastically from that of tungstate. The effect of cystine on ALP activity is practically negligible under the assay conditions used and H_2O_2 as such had no observable effect.

Discussion

The importance of pH for the successful synthesis of peroxotungsten compounds has been emphasized in the literature

[20–22]. The essential parameters for achieving success of the synthesis of the oxo-bridged dinuclear peroxotungsten complexes $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$ (A = Na or K)] were the use of the acidic medium and proper choice of the ancillary ligand. Cysteine is a major metal binding site in proteins which undergo oxidation to the disulfide form, a reaction which may be catalyzed by metal ions or other oxidant species present. Oxidation of cysteine to cystine during the course of the reaction in the present case, is not really unexpected under the reaction conditions used especially in presence of abundant H_2O_2. Depending on the pH of the reaction solution and the nature of the metal, an amino acid such as cystine provides several alternative coordination sites to the metal, viz., terminal amino and carboxylate as well as the S atoms of the -SS- group [29, 30, 32–34]. Thus the chosen amino acid can act as mono, bi or tridentate ligand with different combinations of donor atoms and can occur in complexes in either neutral zwitterionic form or anionic form. In addition, they are known to form bridges between metal atoms leading to the formation of dinuclear or polynuclear structures [29, 32–34]. In the present study, the low pH apparently favored the co-ordination of cystine, in its neutral zwitterionic form to the two W(VI) centers through carboxylate groups thereby stabilizing the $[W_2O_3(O_2)_4]^{2-}$ moiety and leading to the synthesis of the desired complexes **1** and **2**. This is probably the first known example of dimeric oxo-bridged complex isolated into solid state having a co-ligand other than water [20–23]. The fact that such a compound could so far be isolated only with cystine as heteroligand suggests that bridging cystine ligand may have a role in stabilizing the product.

The high stability of the compounds in solution at acidic as well as physiological pH is likely to be a consequence of the additional stability imparted by the bridging heteroligand. It is interesting to note that the synthesized peroxotungstate complexes serve as substrates, although poor ones, for catalase. The enzyme leads to degradation of the compounds at a much slower rate compared to H_2O_2, the natural substrate of catalase. The compounds efficiently oxidized GSH, the major nonprotein thiol in living cells which plays the role of cellular reducing agents and antioxidant [35], to GSSG. However, the observation that only two of the peroxo groups out of 4 peroxides present per molecule of the complex participate in oxidation, defies explanation at this stage.

Alkaline phosphatase is a membrane-bound zinc metalloenzyme. The probable functions assigned to the enzyme include: phosphohydrolysis of organic phosphomonoesters of low molecular mass; phosphotransferase activity and protein phosphatase activity. The maximum activity is shown at pH 8 or above. The reactions catalysed by the enzyme possibly involve an enzyme-phosphate intermediate.

Phosphatases are, in general, inhibited by oxyanions such as vanadate [10–14], molybdate and tungstate [5, 8]. Such inhibition is attributed to the formation of pentaco-ordinated or hexaco-ordinated structures which are often described as phosphate analogue [5, 7–13]. In the present investigation, it is significant to note that the title compounds with heptacoordinated metal centers induce strong inhibitory effect upon the phosphatase activity. Although individually cystine, tungstate and peroxotungstate inhibited ALP activity to varying degrees, it is the cystine containing peroxotungsten complex which turned out to be the most potent inhibitor. As evident from the IC_{50} values and V_0/V_i ratios, even if we take into account the presence of two peroxotungstate units per molecule of the compound, inhibitor potency of the compounds are much higher than expected from equivalent concentrations of tungstate or pW. The inhibitory effect of the complexes may be attributed to some stabilizing factors other than structural analogy with the transition state, or phosphate mimicry. It is reasonable to expect that redox properties of the complex which is also evident from its ability to oxidize GSH should be one of the factors responsible for making the compound a potent inhibitor of the phosphorylase. This appears possible keeping in view the literature reports [10, 12] documenting the importance of redox properties of peroxovanadium compounds in inhibition of protein phosphatases. Another observation relevant to the present investigation is the one made by Crans et al that some 6 and 7 coordinated vanadium and peroxovanadium compounds were capable of inhibiting phophatases [12] from which it was concluded that vanadium compounds do not need to be five-coordinated to be reasonable inhibitors. However, due to the complexity of the reaction and species involved in the present study, we refrain from drawing any conclusion regarding cause of the effect of title compounds on the phosphatase activity. Studies involving effect of other well-defined and stable peroxo-metallates in biogenic ligand environment which remain intact in solution, on the activity of the phosphatases combined with speciation analysis are likely to help in establishing structure-activity correlation of the compounds and in gaining an insight into the pathways involved in such enzyme inhibitory processes. This aspect is currently being studied in our laboratory.

In conclusion, the present results demonstrate that it is possible to isolate the oxo-bridged tetraperoxotungstate(VI) species formed in solution into solid state, through complexation with cystine under appropriate experimental conditions. One of the important findings of the present investigation which may be of clinical relevance is the high stability of the compounds at acidic pH. This may be significant in view of the observation made by Shisheva *et al.* [36] that peroxovanadate, when administered orally was ineffective in inducing normoglycemia in STZ-rats probably because it could not survive the strong acidity of the stomach. Thus the newly synthesized compounds are expected to be of biochemical interest in view of their properties such as their stability toward

decomposition in solution of a wide range of pH values, their being oxidant to GSH and their significant affinity as alkaline phosphatase inhibitor.

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New oxo-bridged dinuclear peroxotungsten(VI) complexes: Synthesis, stability and activity in bromoperoxidation

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Abstract

Two new dinuclear oxo-bridged peroxo complexes of tungsten with coordinated dipeptides of the type, $Na_2[W_2O_3(O_2)_4$(glycyl-glycine)$_2]$ $3H_2O$ (1) and $Na_2[W_2O_3(O_2)_4$(glycyl-leucine)$_2]$ $3H_2O$ (2) have been synthesized from the reaction of H_2WO_4, 30% H_2O_2 and the respective dipeptide at pH ca 2 5 Synthesis of the compounds, in addition to pH, is sensitive to reaction temperature and concentrations of the components The compounds were characterized by elemental analysis, spectral and physico-chemical methods including thermal analysis In the dimeric complexes the two W(VI) centres with edge bound peroxo groups are bridged by an oxo group The dipeptides occurring as zwitterions bind the metal centers through O (carboxylate) atoms leading to hepta co-ordination around each W(VI) Thermal stability of the compounds as well as their stability in solution were determined The compounds are highly stable toward decomposition in solutions of acidic as well as physiological pH These compounds, besides another similar dimeric compound $Na_2[W_2O_3(O_2)_4$(cystine)$]$ $4H_2O$ (3) efficiently oxidized bromide to a bromination competent intermediate in phosphate buffer at physiological pH, a reaction in which only two of the peroxide groups of the complex species were found to be active The complexes could also mediate bromination of organic substrate in aqueous-organic media

Keywords Oxobridged peroxotungstate, Heteroligand diperoxotungsten(VI), Dinuclear peroxotungstate, Peptide containing peroxotungstate, Bromide oxidant, Peroxidative bromination

1. Introduction

The discovery of tungstoenzymes [1,2] and the fascinating findings that peroxotungstates (pW), like vanadates and peroxovanadates (pV), exhibit insulin like properties [3] and were capable of inhibiting activity of protein phosphatases [3–8] have dramatically enhanced the awareness of importance of tungsten and its compounds in biology Among tungsten compounds, peroxo complexes have been for the past several years object of intense investigation, due to their application as an important class of stoichiometric or catalytic oxidizing and oxo-transfer agent in a variety of organic oxidations [9–14]

In spite of many reports dealing with the synthesis and structure of heteroligand diperoxotungsten compounds [12,15–19], there appears to be a paucity of information on synthesis of complexes with $[W_2O_3(O_2)_4]^{2-}$ moiety stabilized by a heteroligand other than H_2O [12,15–19] The dimeric species have been known to exist in a solution of tungstate and excess peroxide at pH $\leqslant$ 5 and are believed to be the species responsible for tungstate catalyzed oxidations by H_2O_2 [12,15] Moreover, reports on synthetic peroxo-W(VI) complexes with co-ordinated biogenic ligands are very few [12,17,20] and there seems to be no available examples of peroxo compounds of tungsten containing peptide as ancillary ligand We have recently synthesized oxobridged tetraperoxo complexes with co-ordinated cystine, $A_2[W_2O_3(O_2)_4$(cystine)$]$ $4H_2O$ (A = Na or K), which exhibited very interesting biochemical and redox properties [20] Pertinent here is to mention that a series

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of peroxo-bridged compounds with amino acids and peptides serving as co-ligands, synthesized by us, proved to be powerful oxidant of bromide at near neutral pH [21–23].

Bromination of organic substrates, particularly aromatics, has been attracting considerable contemporary interest [24–31] mainly due to the commercial importance of such compounds [32]. Since the traditional bromination methods require the use of elemental bromine and solvents which are environmentally hazardous [33], there has been a continued search for alternative benign catalytic systems which can mimic the biological bromoperoxidase in the synthesis of brominated organics. Bromoperoxidases (VBPO), involved in the biosynthesis of brominated marine natural products, catalyze bromination by using H_2O_2 and bromide salts instead of Br_2 [34,35]. A large number of model systems mimicking the enzymatic activity have been developed [35–41], focusing mainly on vanadium and peroxovanadium compounds, particularly to elucidate the mechanistic details of the enzyme function which is yet to be fully understood. Contrary to natural V-BPO which is most efficient at pH 5.5–7 several model complexes were found to be catalytically active in acid medium [35–38, 40–42] which limits their utility as effective catalyst. By itself, H_2O_2 is capable of oxidizing bromide in highly acidic medium (pH < 3) but is ineffective in solution at pH > 5.0. Oxidation of bromide by hydrogen peroxide has been reported to be catalyzed by tungstate(VI) systems in acidic medium [42]. A tungstate-exchanged layered double hydroxide has also been studied as a heterogeneous catalyst in oxidative bromination of olefines by H_2O_2system [31,32]. However, examples of well-defined synthetic heteroligand peroxotungstate compounds displaying activity in oxidative bromination is scanty in literature [43] despite the number of such compounds known.

In view of the above observations in the present work we endeavored to establish rational synthetic routes to new oxo-bridged peroxotungsten complexes containing dipeptide as co-ligand, with appropriate characteristics of solubility and stability. We were particularly interested to examine whether such dinuclear compounds could act as oxidant of bromide with good activity at physiological pH, an essential requirement of a biomimetic model. Reported in this paper are the first synthesis, characterization of oxo bridged dinuclear complexes, $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2]\cdot 3H_2O$ (**1**) and $Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2]\cdot 3H_2O$ (**2**), and studies on their stability and bromination activity. We have also investigated the bromination activity of the complex, $Na_2[W_2O_3(O_2)_4(cystine)]\cdot 4H_2O$ (**3**), synthesized previously by us.

2. Materials and methods

The chemicals used were all reagent grade products. The sources of chemicals are given below: sodium tungstate, cysteine (CDH, New Delhi, India); tungstic acid, (Himedia Laboratories, Mumbai, India); glyclyl-peptides (Sigma–Aldrich Chemical Company); hydrogen peroxide (30%) (Ranbaxy, New Delhi, India); phenol red (from *Aspergillus niger*); potassium bromide, potassium dihydrogen phosphates, sodium and potassium hydroxides (SD fine chemicals, Mumbai, India). The complex, $Na_2[W_2O_3(O_2)_4(cystine)]\cdot 4H_2O$ (**3**) was prepared by methods reported earlier [20]. The water used for solution preparation was deionised and distilled.

2.1. Synthesis of peroxotungsten complexes, $Na_2[W_2O_3(O_2)_4(glyl\text{-}gly)_2]\cdot 3H_2O$ (1) and $Na_2[W_2O_3(O_2)_4(glyl\text{-}leu)_2]\cdot 3H_2O$ (2)

In a typical reaction H_2WO_4 (0.25 g, 1.0 mmol) was added to 10 ml of 30% H_2O_2 (88.2 mmol) contained in a 250 ml beaker. The mixture was kept in an ice bath and stirred slowly until all the solids dissolved and a clear solution was obtained. To this solution dipeptide (1.0 mmol) was added. The pH of the clear solution was recorded to be 1.6. The pH of the reaction mixture was adjusted to ca. 2.5 by adding sodium hydroxide (46.0 mmol). On adding pre-cooled acetone (ca. 50 ml) to the above solution under continuous stirring, a white pasty mass separated out. After allowing it to stand for about 15 min in the ice bath, the supernatant liquid was decanted and the residue was treated repeatedly with acetone under scratching until it became a microcrystalline solid. The product was separated by centrifugation, washed with cold acetone and dried in vacuo over concentrated sulfuric acid. In the solid state these complexes were found to be stable for several weeks stored dry at <20 °C but tended to be hygroscopic at ambient conditions and decomposed in a few days.

Anal. Calc. for $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2]\cdot 3H_2O$ (**1**): Na, 5.06; W, 40.52; O_2^{2-}, 14.09; C, 10.57; N, 6.16; H, 2.20. Found: Na, 4.98; W, 40.13; O_2^{2-}, 13.86; C, 10.35; N, 5.90; H, 2.23%. Yield: approximately 38%.

Anal. Calc. for $Na_2[W_2O_3(O_2)_4(gly\text{-}Leu)_2]\cdot 3H_2O$ (**2**): Na, 4.50; W, 36.07; O_2^{2-}, 12.54; C, 18.82; N, 5.49; H, 3.52. Found: Na, 4.42; W, 35.88; O_2^{2-}, 12.32; C, 18.76; N, 5.40; H, 3.20%. Yield: approximately 37%.

2.2. Elemental analysis

The compounds were analyzed for C, H, and N at the Regional Sophisticated Instrumentation Centre (RSIC), North Eastern Hill University, Shillong, India. The total peroxide content was determined by adding a weighed amount of the compound to a cold solution of 1.5% boric acid (W/V) in 0.7 M sulfuric acid (100 ml) and titration with standard Cerium(IV) solution [44]. Tungsten was determined gravimetrically [45] as $BaWO_4$. The values are given as % by weight of the compounds from which the ratios of W:peroxide are derived.

2.3. Physical and spectroscopic measurements

Spectra in the visible and ultraviolet region were recorded in a Cary Model Bio 100 spectrophotometer,

equipped with a peltier controlled constant temperature cell. The absorbance values are denoted as A_{592} at the wavelength indicated. The infrared (IR) spectra were recorded with samples as KBr pellets in a Nicolet Model Impact 410 FT-IR spectrophotometer and also in a Perkin–Elmer Model 983 spectrophotometer. The spectra were recorded at ambient temperatures by making pressed pellets of the compounds. The ^{1}H NMR spectra were recorded in deuterated chloroform in a Varian EM-390 90 MHz spectrophotometer using TMS as the internal standard. HPLC analyses were performed using a Waters Tm 2487 dual λ detector and assayed at fixed wavelengths using a C_{18} column (Nova-Pak C_{18}, 3.9 × 150 mm, Waters). Magnetic susceptibilities of the complexes were measured by the Gouy method, using Hg[Co(NCS)] as the calibrant. Molar conductance measurements were made at ambient temperature using Systronics Conductivity meter 306. Thermo gravimetric analysis was done in Mettler Toledo Star system at a heating rate of 5 °C/min under the atmosphere of nitrogen using aluminum pan.

2.4. Measurements of bromination activity in solution

The method of de Boer et al. [46] of introducing four bromine atoms into the molecule of phenol red (ε^{433} mmol = 19.7) to form bromophenol blue (ε^{592} mmol = 67.4) was used to measure bromination activity. Phenol red acts as an efficient trap of active bromine species without influencing the rate of reaction until it is exhausted. The reaction mixture contained phosphate buffer (50 mM, pH 5.5), KBr (0.5 M) and phenol red (0.1 mM). The redox activity was tested by adding a measured amount of aliquot from solution of the synthesized compound (1 mM), and by monitoring the possible change in absorbance at 592 nm at 30 °C. The volume of the reaction mixture was kept at 25 ml. Aliquots were transferred to the spectrophotometer immediately after mixing.

2.5. Bromination of organic substrates and product analysis

In a representative procedure, organic substrate (0.5 mmol) was added to a solution of acetonitrile: water (1:1) (3 ml) containing KBr (1.5 mmol). A weighed amount of solid peroxotungstate complex **1** (0.25 mmol) was then added to the reaction mixture at room temperature under continuous stirring. The stirring was continued for ca. 1 h. Reaction products as well as unreacted organic substrates were then extracted with diethyl ether and dried over anhydrous Na_2SO_4. Products were then separated by TLC and HPLC. ^{1}H NMR spectroscopy and melting point determinations were made to interpret the products.

In order to isolate the tungsten species remaining after the completion of the reaction, the following method has been adopted. After extraction of the organic reaction product, the aqueous part of the reaction mixture was transferred to a 250 ml beaker. Keeping the solution in an ice bath, pre cooled acetone was added with constant stirring until a colorless pasty mass separated out. After allowing it to stand for 10 min in an ice bath, the supernatant liquid was decanted off and the residue was treated repeatedly with acetone under scratching until it became white microcrystalline solid. The product was separated by centrifugation, washed with cold acetone and dried in vacuo over concentrated sulfuric acid.

3. Results and discussion

3.1. Synthesis and characterization

The reaction of tungsten with H_2O_2 is highly sensitive to pH and it is known that a number of peroxotungsten species are formed in solution with slight variation of pH of the reaction medium [12,15]. The methodology for the successful synthesis of the white microcrystalline dinuclear peroxotungstate compounds **1** and **2** was based on the reaction of H_2WO_4 with 30% H_2O_2 and the respective ligand in an aqueous acidic medium. Since one of our primary aims was to isolate complexes with a $[WO(O_2)_2]_2O$ moiety which has been reported to exist in an acidic solution [15], the pH of the reaction medium was strategically maintained at <3. Thus the use of acidic pH and choice of the ancillary ligand appeared to be essential parameters for achieving success of the synthesis of the dimeric title compounds. The factors such as maintenance of required time and temperature at $\leqslant$4 °C were found to be equally important for achieving the desired syntheses. We have earlier synthesized such oxo-bridged dimeric compounds, $A_2[W_2O_3(O_2)_4(\text{cystine})]\cdot 4H_2O$ (A = Na and K) by adopting a similar synthetic strategy [20]. Our attempts to isolate analogous dinuclear compounds in the presence of some other ligand systems such as proline, glutamic acid, asparatic acid, thioglycolic acid etc. were unsuccessful. At a relatively higher pH of ca. 5.5 and using Na_2WO_4 as source of tungsten(VI), monomeric diperoxotungsten complexes were obtained in each of these cases. This suggests that the chosen dipeptide ligands may have a role in stabilizing the products.

The title compounds were diamagnetic in nature as was evident from the magnetic susceptibility measurement in conformity with the presence of tungsten(VI) in each case. From the elemental analysis data, the ratio of 1:2 was obtained for W:peroxide, while W:co-ligand ratio was ascertained to be 1:1 in each of the complexes. The elemental analysis results and molar conductance values obtained from measurement at ambient temperature were in complete agreement with the formulation of the complex species as $Na_2[W_2O_3(O_2)_4(\text{gly-gly})_2]\cdot 3H_2O$ (**1**) and $Na_2[W_2O_3(O_2)_4(\text{gly-Leu})_2]\cdot 3H_2O$ (**2**), respectively.

Each of the title compounds displayed characteristic spectral patterns in the infra-red region involving absorptions due to co-ordinated peroxide, amino acid or dipeptide ligand and lattice water (Table 1). The strong absorption at ca. 960 cm^{-1} was consistent with the presence of a terminally bonded W=O group in each case [15,16]. For the dinuclear W–O–W unit the antisymmetric

Table 1
The structurally significant IR bands of the compounds **1** and **2**

S. no.	Compound	IR bands					
		ν(W=O)	ν(O–O)	$\nu(W_2O)$	$\nu_{as}(W–O_2)$	$\nu_s(W–O_2)$	$\nu_{sym}(W_2O)$
1	$Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2]\cdot 3H_2O$ (**1**)	952	843	770	612	531	410
2	$Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2]\cdot 3H_2O$ (**2**)	949	845	767	613	534	415

and symmetric stretchings were expected in the 750–770 and 500 cm^{-1} regions [15,16], respectively. In the spectra of the complexes **1** and **2**, a prominent band at ca. 770 cm^{-1} and another weak intensity band appearing at ca. 410 cm^{-1} attributable to $\nu_{asym}(W_2O)$ and $\nu_{sym}(W_2O)$ modes gave clear indication of the presence of a bridging oxo group in each of them. The presence of side-on bound peroxo ligand in the compounds, was evident from the observance of the expected ν(O–O), ν_2 and ν_3 modes which involve metal-oxygen stretches of $W–O_2$ vibrations in the range of ca. 850, ca. 610 and ca. 530 cm^{-1}, respectively.

IR spectroscopy is immensely useful and informative for the characterization of heteroligand peroxometal complexes since it provides information not only about co-ordination of peroxo but also ligand groups when compared with the spectra of the free ligands. The characteristic shifts of heteroligand bands that occur upon co-ordination compared to the spectra of the free ligand reveal the bonding sites in the co-ordinated ligands. The spectra of the complexes **1** and **2** exhibited two distinct bands in the range of 1680–1660 cm^{-1} and 1630–1590 cm^{-1} which have been assigned to ν(C=O) (amide) and ν_{as}(COO) of the co-ordinated peptide ligands [47–49]. The position of ν(C=O) band in the complexes almost remained unaltered compared to its position in free ligand which indicated that the amide group was not taking part in co-ordination. The broadening of the band was probably owing to their participation in hydrogen bonding. Co-ordination through N-atom of the amide group was unlikely as evident from the spectra because such co-ordination is known to cause considerable decrease in the peptide carbonyl stretching frequency which was not observed in case of these compounds [50,51]. The ν_s(COO) vibration of the free ligands were observed in the range of 1410–1400 cm^{-1} in the IR spectra [47]. A medium intensity band with some broadening observed in the range of 1395–1405 cm^{-1} region was assigned to ν_s(COO) of the unidentate carboxylate group ($\nu_{as}-\nu_s \approx 300$ cm^{-1}) [47]. The broadening of the band was probably caused by its mixing with the C–N stretching of amide group expected to occur in this region [49]. The spectra showed N–H stretching bands of coordinated peptide residue at 3300–3100 cm^{-1} region as expected from the $-N^{+}H_3$ group. N–H deformation modes (1520–1600 cm^{-1}) in their position and pattern in the spectra of the complexes significantly remained unaltered compared to the free ligand. Based on these observations it may be inferred that the dipeptide ligands, occurring as zwitterions in the complexes, co-ordinate to tungsten(VI) through carboxylate group. The presence of lattice water in each of the complexes **1** and **2** was indicated by the strong ν(OH) absorptions displayed at 3500–3400 cm^{-1}. However, the bending mode of water could not be assigned with certainty as it occurred in the carbonyl frequency region.

Depending on the pH of the reaction solution and the nature of the metal, a ligand like an amino acid or a simple peptide can act as mono, bi or tridentate ligand with different combinations of donor sites, viz., terminal amino and carboxylate groups [52] as well as amide groups in peptides and can occur in complexes in either neutral zwitterionic form or anionic form. In addition, they are known to form bridges between metal atoms leading to the formation of dinuclear or polynuclear structures. In the present study, the low pH apparently favored the co-ordination of dipeptides in their neutral zwitterionic form to the two W(VI) centers through carboxylate groups thereby stabilizing the $[WO(O_2)_2]_2O$ moiety and leading to the synthesis of the desired complexes **1**and **2**. Hydrogen bonding between the side chains is likely to stabilize the complex species in the solid state. Based on these observations a structure of the type shown in Fig. 1 has been proposed for complexes **1** and **2**, which is shown for the glycyl-glycine complex as a representative.

3.2. Stability of the complexes in solution

The stability of the compounds in an aqueous solution of pH 3.5, which is the pH attained by the solution on dissolution of the compounds, has been studied by estimating the peroxide content and molar conductances at different time intervals for any possible change. The investigations revealed that their peroxide content and molar conductance values (261 Ω^{-1} cm^2 mol^{-1} for **1** and 257 Ω^{-1} cm^2 mol^{-1} for **2**) remained unaltered for over a period of 12 h and oxygen was not released on dissolution of the complexes in

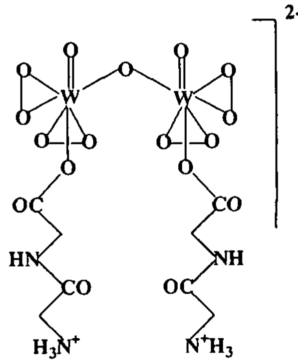

Fig. 1. Proposed structure of the dinuclear peroxotungstate compounds shown with $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2]$ $3H_2O$ as representative.

water. The compounds were also found to be stable at pH 7.0 as well as at pH 8.0. The high stability of the compounds in solution at acidic as well as physiological pH is likely to be a consequence of the additional stability imparted by the heteroligands.

3.3. Thermal analysis

Thermo gravimetric analysis data of the compounds provided crucial information regarding composition of the compounds and their thermal stability. It has been evident from the TG and DTG curves (Fig. 2) that after the initial dehydration, the compounds **1** and **2** undergo multistage decomposition up to a final decomposition temperature of 550 °C.

The first stage of decomposition occurs at 40–100 °C with the liberation of the outer sphere water molecules from the complexes (Table 2). The observed weight loss of 5.4% and 5.0% for the complexes **1** and **2**, respectively are in good agreement with the calculated values (Table 2). Another decomposition stage observed in the temperature region of 110–200 °C for complex **1** and 90–160 °C for complex **2** with a corresponding weight loss of 13.2% and 11.5%, respectively is characteristic of complete loss of co-ordinated peroxo groups from the complexes [17]. It seems likely that the nature of the heteroligand has a specific effect on the thermal stability of the complexes, though not a large one, as reflected in the variation in their decomposition temperatures with respect to the loss of peroxide. Absence of peroxo group in the decomposition product

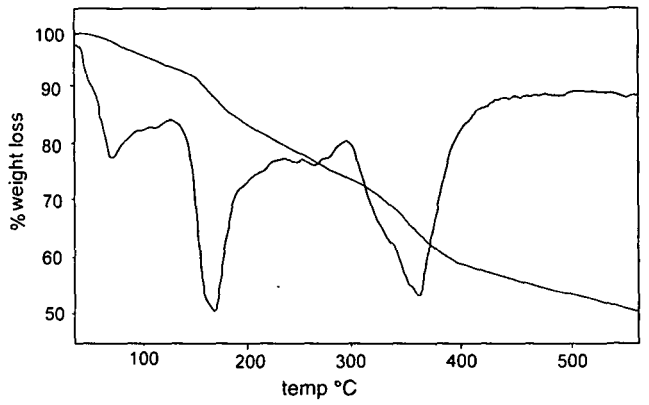

Fig. 2 TG and DTG plot of $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2]\ 3H_2O$ (**1**).

was confirmed from the infrared spectral analysis. Mention must be made that some peroxotungstate and peroxomolybdate compounds containing amino acids as co-ligands were reported to behave in a similar manner losing their peroxide completely on heating [17].

The total weight loss which occurred during the course of the overall decomposition process on heating the compounds up to a final temperature of 550 °C was recorded to be 48.08% for complex **1**, which is in good agreement with the theoretically calculated value of 49.15% for the complete loss of the components viz, lattice water, co-ordinated peroxide, and the glycyl-glycine ligands. The remaining dark brown sticky residue after heating up to 550 °C was found to be a hydrated oxotungstate species as indicated by the IR spectrum which displayed the characteristic ν(W=O) and ν(OH) absorptions and was devoid of bands attributable to peroxo and the dipeptide ligands of the original compounds.

3.4. Redox activity of the complexes in bromination reaction

The bromination of phenol red to its tetra brominated product, bromophenol blue was used to measure the bromination activity of the complexes **1–3** in solution. Addition of freshly prepared aqueous solution of each of the compounds **1–3**, at concentrations indicated (Table 3), to the standard reaction of bromide in phosphate buffer with phenol red as trap for oxidized bromine resulted in gradual color change of the solution from yellow to blue. The spectrum recorded showed a peak at A_{592} characteristic of the product bromophenol blue and a decrease in absorbance of the peak at A_{433} due to loss of phenol red (Fig. 3). The data in Table 3 show that the dinuclear complexes **1–3** possess bromination activity.

A similar reaction when carried out in absence of phenol red displayed a peak at 262 nm with a shoulder at 237 nm on addition of solutions of compounds. Addition of phenol red to this solution resulted in the decrease in A_{262} nm and a peak at 592 nm appeared indicating the formation of bromophenol blue. The 262 nm peak, therefore, represents a bromination competent oxidized species of bromide, probably an equilibrium mixture of BrOH, Br_2 and Br_3^- as proposed earlier [34].

It was of significance to note that the bromination activity of each of the dimeric tetraperoxo complexes with two oxo-bridged diperoxotungsten units were recorded to be limited

Table 2
Thermal decomposition of peroxotungstate(VI) complexes **1** and **2**

Compound	Temperature range (°C)	Weight loss			
		H_2O (%)		O_2^{2-} (%)	
		Found	Calc.	Found	Calc.
$Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2]\cdot 3H_2O$ (**1**)	40–100	5.4	5.9		
	110–200			13.2	14.1
$Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2]\cdot 3H_2O$ (**2**)	40–90	5.0	5.2		
	100–160			11.5	12.5

Table 3
Bromination of phenol red with peroxotungstate complexes **1–3**

Compounds	Conc (mM)	Rate of bromine transfer		Total bromine transfer (extrapolated to 1 mM compound)
		ΔA_{592}/min	μM Br/min	mM Br/mM compound
1 $Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2]$ $3H_2O$ (**1**)	0 1	0 16	9 5	2 01
2 $Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2]$ $3H_2O$ (**2**)	0 1	0 18	10 9	1 88
3 $Na_2[W_2O_3(O_2)_4(cystine)]$ $4H_2O$ (**3**)	0 1	0 19	11 2	1 94

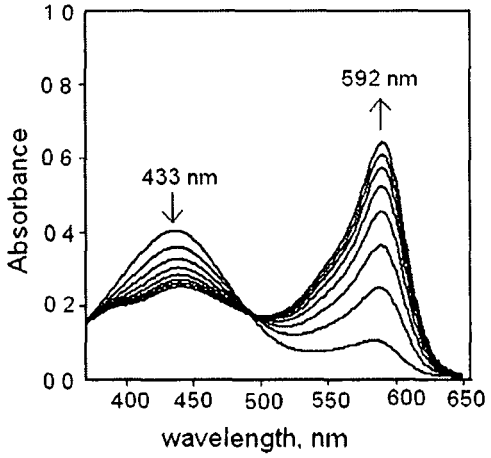

Fig 3 Bromination activity with dinuclear peroxotungstate compound **1** The spectrum recorded immediately after adding the compound solution to the reaction mixture Spectral changes at 2 min interval The reaction mixture contained phosphate buffer (0 05 M, pH 5 5), KBr (0 5 M), phenol red (0 1 mM) and compound **1** (0 05 mM)

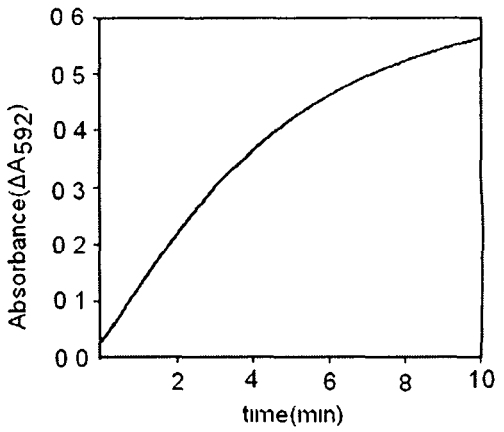

Fig 4 The increase of absorbance at 592 nm indicating the rate of bromination with compound **1** The reaction mixture contained phosphate buffer (0 05 M, pH 5 5) KBr (0 5 M), phenol red (0 1 mM) and compound **1** (0 05 mM)

to ca 50% of that expected on the basis of the total number of peroxo groups present in the complexes (Table 3) Decrease or increase in concentrations of the compound, substrate or KBr in the reaction solution had no effect on this feature The consistently observed stoichiometry of 2 1 for bromine transferred peroxotungsten compound cause us to infer that only one of the peroxo groups of a diperoxotungsten moiety of the tetraperoxotungsten complex would be active in bromide oxidation

The effect of H_2O_2 on the bromination reaction under standard assay conditions was tested While the initial addition of H_2O_2 (0 5 mM) to the reaction solution had no observable effect on the initial rate of bromination (Fig 4), it was quite intriguing to note a revival of the bromination activity on addition of H_2O_2 (0 5 mM), after bromination to a spent reaction mixture which contained excess bromide and substrate It is thus plausible that an inactive tungsten intermediate formed after completion of the bromination process probably combines with peroxide in presence of excess H_2O_2 to regenerate the respective starting dimeric tetraperoxo tungstate complex giving rise to a catalytic cycle Exogenous hydrogen peroxide is therefore required to maintain the tungsten complexes in their tetraperoxo forms in order to obtain a catalytic cycle

The bromination activity of the compounds was surprisingly, found to be maximum at pH 7 5, unlike in the case of peroxovanadate catalyzed oxidative bromination where the rate was reported to increase monotonously with increasing acidity of the reaction medium [53] We have obtained the data in Table 3 at pH 5 5 since the use of this method is limited to pH range near 5 0 in order to achieve conversion from phenol red (pK_a 7 9) to bromophenol blue (pK_a 4 0) Omission of phosphate buffer from the reaction medium did not alter the bromination activity of the complexes This indicated that the presence of phosphate was not essential for such activity of the peroxotungsten compounds

Efficacy of the complexes in mediating bromination of organic substrates in presence of bromide in aqueous-organic media has been explored Bromination of several activated aromatics into their corresponding bromo-organics took place in moderate yields in presence of the dinuclear pW complexes (Table 4) at ambient temperature Reactions were carried out in the absence of buffer Preferential bromination at either *ortho* or *para* positions of the aromatic ring leading to mono substitution indicate an electrophilic bromination mechanism That the brominating species was 'Br^+' and not a '$Br^\cdot$' in these reactions was further evident from the ring substituted products obtained from the substrate, 2-methoxy toluene (Table 4) Bromination through radical reaction would have produced benzyl bromide instead of bromo-methoxy toluene

In order to ascertain the nature of the inactive intermediate formed in solution and to rationalize the reaction sequence the product isolated from the aqueous extract

Table 4
Bromination of organic substrates mediated by compound 1

Substrate	Product	% Yield
Aniline	4-bromoaniline	52
	2-bromoaniline	35
o-Nitroaniline	4 bromo-2-nitroaniline	61
	2-bromo-6-nitroaniline	30
m Nitroaniline	2-bromo-5-nitroaniline	56
	4-bromo-3-nitroaniline	30
p-Nitroaniline	2-bromo-4-nitroaniline	85
o-Aminophenol	2-amino-5-bromophenol	79
m-Aminophenol	3-amino-6-bromophenol	60
	3-amino-4-bromophenol	30
p-Aminophenol	4-amino-3-bromophenol	76
Quinol	2-bromo-4-hydroxyphenol	72
o Methoxytoluene	3-bromo-6-methoxytoluene	58
	3-bromo-2-methoxytoluene	34

of the reaction mixture, after completion of the reaction, was subjected to IR and elemental analysis IR spectrum of the compound resembled closely the spectrum of the original starting complex showing the presence of peroxo group, bridging as well as terminal oxo groups and co-ordinated dipeptide Elemental analysis results suggested the presence of one peroxo group per W(VI) indicating the formation of a monoperoxotungsten species It thus appears that the dimeric complex remains intact throughout the course of the bromination process where two of its peroxo groups participate, leading to the formation of a dimeric peroxotungsten compound with two heteroligand monoperoxo moieties bonded through an oxo group It is interesting to note that we could also isolate an analogous dimeric mono peroxotungsten species from a separate experiment involving complex **1** (0 5 mM) and KBr (2 0 mM) under standard assay condition with phenol red omitted from the reaction medium This observation is consistent with the proposal which implicates formation of a monoperoxotungsten intermediate which is inactive in bromination

A scheme of reactions, shown in Fig 5, is proposed which satisfactorily describes the principal features of our results The dimeric compounds react with bromide to yield oxidized bromine species, proposed to be an equilibrium mixture of BrOH, Br_2 and Br_3^-, with concomitant formation of dinuclear intermediate which is likely to possess two monoperoxotungsten units with the oxo-bridge retained (reaction a) Transfer of the bromine atom to the substrate AH from the bromination competent oxidised bromine intermediate takes place (reaction b) In the presence of excess H_2O_2 the diperoxo intermediate combines with peroxide to regenerate the starting tetraperoxo ditungstate complex giving rise to a catalytic cycle (reaction c) To us it appears that bromide would attack an edge-bound peroxo group in preference to a hepta co-ordinated tungsten centre as observed in some other redox processes involving peroxo compounds of W(VI) and Mo(VI) [13,14,43]

Formation of an inactive monoperoxo intermediate from a catalytically active diperoxotungsten or molybdenum complex during oxygen-atom transfer reactions med-

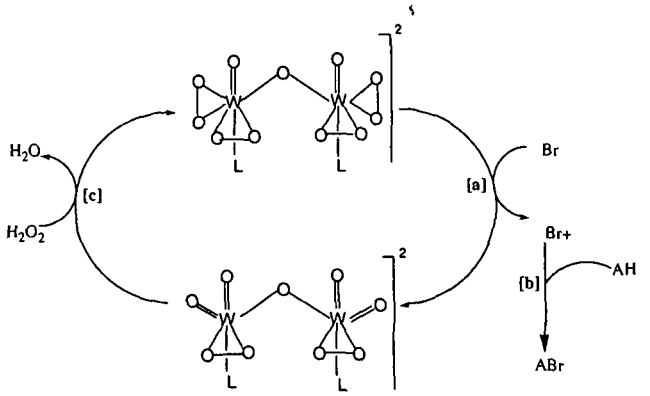

Fig 5 Schematic representation of reactions occurring with dinuclear tetraperoxotungsten(VI) compounds (L = cystine, gly-gly or gly-leu) (a) reaction of the complex with bromide to yield oxidized bromine and an intermediate postulated as a diperoxotungsten species having two monoperoxotungsten units with the oxo-bridge retained, (b) transfer of bromine from the active species to acceptor AH, (c) in presence of excess H_2O_2 the diperoxo intermediate combines with peroxide to regenerate the starting tetraperoxo tungstate complex giving rise to a catalytic cycle No attempt is made to show the exact stoichiometry of the reaction

iated by such complexes is not unprecedented [13,14] A monoperoxo Mo(VI) has been implicated as an intermediate in the mechanisms proposed by Reynolds et al [43] as well as by Butler and co-workers for the Mo(VI) and W(VI) catalyzed bromide oxidations [42] It is also relevant in this context that during one of our previous studies involving redox activity of the complex **3** with respect to reduced glutathione (GSH), only two of the peroxo groups out of four peroxides present per molecule of the complex were found to be involved in oxidation [20] These considerations fortify our proposed mechanism

The observed reaction pattern is in accord with the earlier suggestions that for a peroxotungsten complex to be active in oxidation an oxo-diperoxo configuration may be a prerequisite [13] This feature is however, in contrast to the findings that diperoxo-vanadate compounds with only η^2-peroxo groups in its co-ordination sphere were catalytically incompetent in bromide oxidation at neutral pH [23,53] A dinuclear peroxovanadate with a μ-peroxo group, on the other hand was highly active in bromination at physiological pH [21–23,53] The proposed reaction pathway conferred the status of a bromide oxidant, at physiological pH, on the VOOV group [21–23,53] The mechanisms of bromide oxidation mediated by pW and pV compounds, tested by us, thus appear to be distinctly different It is not completely clear however, as to why a η^2-peroxo group of diperoxotungsten(VI) species is highly reactive in oxidative bromination while a diperoxovanadate species with similar edge-bound peroxo groups is ineffective in bromination at near neutral pH

4. Conclusion

In summary, with the examples of the newly synthesized dinuclear complexes the present investigation has

established that it is possible to isolate the oxo-bridged tetraperoxotungstate(VI) species formed in solution into solid state, through complexation with specific ligands under appropriate experimental conditions A noteworthy observation of the present investigation, which may also be of biochemical importance, is the high stability of the compounds in solution at a wide range of pH values particularly at acidic pH Undoubtedly, the most notable aspect of the present study is the finding that the newly synthesized complexes besides being stoichiometric reagents for bromide oxidation in aqueous medium, also acted as a catalyst for the same reaction when used in conjunction with H_2O_2 at neutral pH Isolation of the diperoxoditungsten(VI) species as reaction intermediate lends support to the proposed mechanism Information generated from the present investigation may find relevance in the context of designing safer biomimetic redox catalysts for organic bromination

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Mononuclear and dinuclear peroxotungsten complexes with co-ordinated dipeptides as potent inhibitors of alkaline phosphatase activity

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Abstract

New molecular peroxotungstate(VI) complexes with dipeptides as ancillary ligands of the type, $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$, dipeptide = glycyl-glycine or glycyl-leucine, have been synthesized and characterized by elemental analysis, spectral and physico-chemical methods including thermal analysis. The complexes contain side-on bound peroxo groups and a peptide zwitterion bonded to the metal centre unidentately through an O(carboxylate) atom. Investigations on certain biologically important key properties of these compounds and a set of dimeric compounds in analogous co-ligand environment, $Na_2[W_2O_3(O_2)_4(dipeptide)_2].3H_2O$, dipeptide = glycyl-glycine and glycyl-leucine, reported previously by us revealed interesting features of the compounds. Each of the compounds despite having a 7 co-ordinated metal centre exerts a strong inhibitory effect on alkaline phosphatase activity with a potency higher than that of the free dipeptide, tungstate or peroxotungstate. The compounds exhibit remarkable stability in solutions of acidic as well as physiological pH and are weaker as substrate to the enzyme catalase, compared to H_2O_2. The mononuclear and dinuclear peroxotungsten compounds are efficient oxidants of reduced glutathione (GSH), a reaction in which only one of the peroxo groups of a diperoxotungsten moiety of the complexes was found to be active.

Keywords: *ALP inhibitor, peptide containing peroxotungstate, GSH oxidant, substrate to catalase, alkaline phosphatase, inhibition*

Introduction

Peroxotungsten complexes have been receiving continued importance mainly owing to their application as an important class of stoichiometric or catalytic oxidizing and oxo-transfer agent in a variety of organic oxidations [1–8]. There has been a revival of interest in peroxo-tungsten systems since it has been demonstrated that tungstates and peroxotungstates (pW) present in a solution of W-H_2O_2, like vanadate and peroxovanadates (pV), were capable of inhibiting the hydrolysis of phosphoproteins [9–13] and mimic the insulin bioeffects in rat adipocytes [10]. Moreover, with the exciting discovery of several tungstoenzymes in recent years biological relevance of tungsten and its compounds has finally been recognized [14,15]. Recent findings on the ability of the compounds of tungsten such as polyoxotungstates and silicotungstates to show antiviral activity [16,17] and to be potent inhibitors of HIV reverse transcriptase and RNA-dependant DNA polymerase [17] are exciting contributions to the current knowledge of biochemical importance of the metal and its compounds. The importance of enzyme inhibition as a mode of action for inorganic drugs is being realized in recent years and is an area needing exploration [16].

Although much research has been performed on the potential usage of compounds of vanadium as therapeutic antidiabetic agents [18–21], the detailed mechanism by which vanadates and peroxovanadates induce their insulin-mimetic effect or inhibit enzyme functions is yet to be fully understood [22–24].

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A definite correlation was found to exist between abilities of vanadate and pV to inhibit protein phosphatases and their in vivo insulin mimetic activities [22–24]. However, most of the synthetic pV compounds tested for their various biochemical effects suffer from disadvantage of being hydrolytically unstable and this limits their utility as therapeutic agents [18,19,24]. It is notable in this context that peroxotungstates, formed in a solution of W-H_2O_2 not only exhibited insuline-like behaviour but were also found to be stable in solution of a wide range of pH values [10]. Surprisingly, despite these important findings to the best of our knowledge there has been no information available involving the effect of well-defined synthetic heteroligand peroxotungsten compounds on activity of different enzymes including phosphatases [16], until we reported our observations on the phosphatase inhibitory effect of the dinuclear pW compounds, $A_2[W_2O_3(O_2)_4(cystine)].4H_2O$ (A = Na or K), with a potency significantly higher than that of the free cystine, tungstate, or peroxotungstate [25]. Most importantly, these complexes with co-ordinated cystine, were found to be highly stable in solution of acidic as well as alkaline pH, served as a weak substrate to catalase, and efficiently oxidized GSH to GSSG [25].

As a direct sequel to this and in order to gain a better insight into the afore mentioned aspects of pW systems, we considered it imperative to generate further information regarding biologically relevant properties of new monomeric and dimeric pW compounds in biogenic co-ligand environment. Pertinent here is to mention that work on pW compounds containing biogenic species such as amino acids and peptides as ancillary ligands have so far received scant attention [4,26,27]. We have already gained an access to first peptide containing pW compounds of the type, $Na_2[W_2O_3(O_2)_4(dipeptide)_2].3H_2O$, dipeptide = glycyl-glycine (1) and glycyl-leucine (2), which proved to be efficient oxidant of bromide at physiological pH [26], an essential requirement of biomimetic model. In the present study therefore, we have been specifically interested to obtain monomeric pW analogues of these compounds, which would also enable us to investigate whether monomeric and dimeric pW compounds in similar co-ligand environmrnt would exhibit similarities in their redox and solution properties.

In this paper, we report the synthesis and characterization of new molecular peroxo compounds of W(VI)), $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$, dipeptide = glycyl-glycine (3) and glycyl-leucine (4). The thermal stability of the newly synthesized compounds as well as their stability towards decomposition in solution have been examined and compared with their dimeric analogues. We have investigated the redox activity of the mononuclear and dinuclear compounds 1–4 with respect to GSH and their interaction with catalase. Effects of the two types of pW complexes 1–4 upon alkaline phosphatase (ALP) activity have been determined.

Materials and methods

Chemicals and solutions

The sources of chemicals are given below: Catalase, alkaline phosphatase from rabbit intestine, *p*-nitrophenyl phosphate (p-NPP) and glycyl-peptides (Sigma-Aldrich Chemical Company, New Delhi); sodium and potassium tungstates, (CDH, New Delhi,India); hydrogen Peroxide (30%) (Ranbaxy, New Delhi, India); dithionitrobenzoic acid (DTNB), glutathione (Himedia laboratories, Mumbai, India); glycine, potassium dihydrogen phosphates, sodium and potassium hydroxides, magnesium chloride (SD Fine Chemicals, Mumbai, India). The complexes $Na_2(W_2O_3(O_2)_4(gly\text{-}gly)_2(.3H_2O$ (1) and $Na_2(W_2O_3(O_2)_4(gly\text{-}leu)_2(.3H_2O$ (2) were prepared by methods reported earlier [26]. The water used for solution preparation was deionized and distilled.

Synthesis of monomeric peroxotungstate complexes, $[WO(O_2)_2(glycyl\text{-}glycine)(H_2O)].3H_2O$ (3) and $[WO(O_2)_2(glycyl\text{-}leucine)(H_2O)].3H_2O$ (4):

The procedure adopted for the synthesis is common to both complexes. This consisted of gradual addition of 5 mL H_2O_2 (30% solution, 44 mmol) to a mixture of H_2WO_4 (0.5 g, 1.5 mmol) and peptides at a molar ratio of W: dipeptide of 1:1 with continuous stirring. Keeping the temperature below 4°C in an ice bath, the mixture was stirred for ca. 5 min until all solids dissolved. At this stage the pH of the solution was recorded to be ca. 1.5. The pH of the reaction mixture was raised up to 5.5 by adding NaOH solution (0.1 M) dropwise. On adding pre-cooled acetone (about 50 mL) to the above solution under continuous stirring a colorless pasty mass separated out. After allowing to stand for about 10 min in the ice bath, the supernatant liquid was decanted off and the residue was treated repeatedly with distilled acetone under scratching until it became a white microcrystalline solid. The product was separated by centrifugation, washed with cold distilled acetone and dried in vacuo over concentrated sulfuric acid.

Anal. Calc. for $[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$ (3):. W, 39.40; O_2^{2-}, 13.70; C, 10.27; N, 5.99; H, 3.42. Found: W, 38.85; O_2^{2-}, 13.56; C, 10.57; N, 5.73; H, 3.12. Yield: approximately 45%

Anal. Calc. for $[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$ (4):. W, 35.11; O_2^{2-}, 12.21; C, 18.32; N, 5.34; H, 4.58. Found: W, 35.28; O_2^{2-}, 12.12; C, 18.25; N, 5.40; H, 3.86. Yield: approximately 48%

Elemental analysis. The compounds were analyzed for C, H, and N at the Regional Sophisticated Instrumentation Centre (RSIC), North Eastern Hill University, Shillong, India. The total peroxide content was determined by adding a weighed amount of the compound to a cold solution of 1.5% boric acid (W/V) in 0.7 M sulfuric acid (100 mL) and titration with standard Cerium (IV) solution [28]. Tungsten was determined gravimetrically [29] as $BaWO_4$. The values are given as % by weight of the compounds from which the ratios of metal: peroxide are derived.

Physical and spectroscopic measurements. The IR spectra were recorded with samples as KBr pellets in a Nicolet model 410 FTIR spectrophotometer. The spectra were recorded at ambient temperature by making pressed pellets of the compounds. Spectroscopic determinations of the initial rate of ALP catalyzed hydrolysis of p-NPP and oxidations of GSH were carried out in a Cary model Bio 100 spectrophotometer, equipped with a peltier controlled constant temperature cell. The absorbance values were denoted as e.g. A_{405} at the wavelength indicated. Thermogravimetric analysis was done in Mettler Toledo Star system at a heating rate of 5°C/min under the atmosphere of nitrogen using aluminum pan.

Stability of complexes 3 and 4 in solution. Stability of the compounds in distilled water at pH ca. 5, the natural pH of the solutions of the compounds in water, was studied by estimating the peroxide content in aliquots drawn from a solution of the compounds (0.1 mM) at different time intervals by the method described above (Figure 2). As a measure of stability of the compounds in solution change in absorbance of their electronic spectral bands at ambient temperature were recorded at 30 min gap for a period of 12 h. Stability of the compounds at pH 7.0 or 8.0 was measured similarly in compound solution (0.1 mM) in phosphate buffer (50 mM, pH 7.0 or 8.0). In order to measure the stability of the compounds at acidic pH citrate buffer was used (pH 3.4).

Effect of catalase on the complexes 1– 4. The effect of catalase on complexes was studied by estimating the peroxide content of the compounds in a solution containing catalase at specified time intervals (Figure 2). The test solution contained phosphate buffer (50 mM, pH 7.0) and catalase (40 μg/mL). The volume of the reaction solution was kept at 25mL. The solution was incubated at 30°C. The compound was then added to the test solution and aliquots of 5 mL were pipetted out and titrated for peroxide content after stopping the reaction by adding it to cold sulfuric acid (0.7 M, 100 mL) at time 5, 10, 15, 20, 25 and 40 min of starting the reaction. Three concentrations of peroxo compounds (0.05, 0.1, 0.2 mM) were tested.

Measurement of redox activity in solution. To a reaction mixture containing GSH (80 μM) and phosphate buffer (50 mM, pH 7.0) a measured amount of aliquot from solution of the synthesized compound (1 mM) was added. Following incubation of 10 min, DTNB (160 μM) was added to the solution and the change in absorbance at 412 nm was determined in order to measure the GSH remaining in solution by the method of Ellman [30] using molar extinction of $\varepsilon_{412} = 13{,}600$. Measurements were done by using different concentrations of the compounds, 5.0 and 10.0 μM, in triplicate under same assay conditions.

Measurement of alkaline phosphatase activity. Phosphatase activity was assayed spectrophotometrically by using p-nitrophenyl phosphate (p-NPP) as a substrate. The continuous production of p-nitrophenol (p-NP) was determined at 30°C by measuring absorbance at 405 nm in a reaction mixture containing ALP from rabbit intestine (3.3 μg protein/mL), p-NPP (1 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0). The initial reaction rates were obtained by starting the reaction by adding ALP to the reaction solution, which was pre-incubated for 5 min. The initial reaction rate of p-NPP hydrolysis in the absence of the inhibitors, V_0 was determined which was used as control. The effects of peroxotungstate and other inhibitors were assessed by adding different concentrations (10–100 μM) of each species in the ALP assay. The V_i was obtained as the rate of p-NPP hydrolysis in the presence of variable concentrations of inhibitors under similar experimental conditions. The V_0/V_i ratios were calculated from these values. The concentrations tested for the compounds and each of the other inhibitors were 10, 20, 30, 40, 50 and 75 μM. The IC_{50} values were graphically determined as the half-maximal inhibitory concentration of the inhibitor species giving 50% inhibition. All the assays were performed in triplicate. The data in figures are presented as the means ± SE from three separate experiments.

Results

Synthesis and characterization

The synthesis of monomeric peroxotungsten compounds 3 and 4 has been achieved from the reaction of H_2WO_4 with 30% H_2O_2 and the respective ligand at near neutral pH of ca. 5. The procedure included other essential components such as maintenance of required time and temperature at ≤ 4°C and limiting water to that contributed by 30% H_2O_2 and alkali hydroxide solution.

Table I. Infrared and ultraviolet spectral data of compounds 3 and 4.

Compound	IR peak (cm^{-1})				UV peak (nm)
	ν(W=O)	ν(O-O)	ν_{as}(W-O_2)	ν_s(W-O_2)	
$[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$ (3)	957	875	615	539	254
$[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$ (4)	956	876	593	540	252

The complexes were obtained as white microcrystalline products, hygroscopic in nature. In the solid state they remained stable for several weeks when stored dry in closed containers at temperature < 30°C. The elemental analysis data for the compounds 3 and 4 indicated the presence of two peroxide groups and one peptide ligand per metal centre which could be fitted with the formulation of the complex species as $[WO(O_2)_2(dipeptide)(H_2O)].3H_2O$, dipeptide = glycyl-glycine (3) and glycyl-leucine (4).

The IR spectra of the compounds 3 and 4 gave clear indication of the presence of co-ordinated peroxide, co-ordinated peptide, terminally bonded W=O in each of them. The significant general features are presented in Table I. The occurrence of side-on bound peroxo ligand in these compounds was evident from the observance of the characteristic ν(O-O), ν_{as}(W-O_2) and ν_s(W-O_2) modes, in the vicinity of ca. 870, ca. 610 and ca. 530 cm^{-1}, respectively. The spectra enabled clear identification of ν(W=O) near 960 cm^{-1} arising from terminally bonded W=O group [31,32].

The IR spectra of glycyl-glycine and its compounds have been extensively studied in solution [33–36] as well as in solid state [33]. A clear resemblance was observed between the IR spectral pattern originating from co-ordinated peptides of the monomeric compounds 3, 4 and the corresponding dinuclear peroxotungstates 1 and 2 [26]. The spectra of the complexes 3 and 4, exhibited two distinct bands in the range of 1680 – 1660 cm^{-1} and 1630–1600 cm^{-1} which have been assigned to ν(C=O) (amide) and ν_{as}(COO) of the co-ordinated peptide ligands [33–36]. The position of ν(C=O) band remained almost unaltered compared to its position in free glycyl-glycine (1675 cm^{-1}) or glycyl-leucine (1690 cm^{-1}), which indicated that the amide group was not taking part in co-ordination. Bonding through N-atom of the amide group was unlikely as evident from the spectra because such co-ordination is known to cause considerable decrease in the peptide carbonyl stretching frequency, which was not observed in case of these compounds [37,38]. The ν_s(COO) vibration of the free ligands were observed in the range of 1410 cm^{-1} (glycyl-leucine) and 1395 cm^{-1} (glycyl-glycine) in the IR spectra [32]. A medium intensity band with some broadening observed at ca. 1380–1390 cm^{-1} region was assigned to ν_s(COO) of the unidentate carboxylate group ($\nu_{as} - \nu_s \approx 250$ cm^{-1}) [37]. The broadening of the band was probably caused by its mixing with the C–N stretching of amide group expected to occur in this region [36]. The spectra showed N-H stretching bands of coordinated peptide residue at 3300–3100 cm^{-1} regions as expected from the -N^+H_3 group. The rocking modes of -N^+H_3 occurred at ca. 1130 and ca. 1042 cm^{-1}. The presence of water in the complexes was evident from the broad absorption at 3500–3400 cm^{-1}, due to ν(OH). Owing to the presence of lattice water, IR spectral information on ν(OH) and δ(H-O-H) modes are not very significant in so far as the distinction between co-ordinated and lattice water are concerned. Fortunately, a consistent appearance of a medium intensity signal at ca. 725 cm^{-1} attributable to rocking mode of water indicated the presence of co-ordinated water in each of the compounds. The IR spectral data thus suggest that in the complexes 3 and 4, dipeptide ligand occurring as zwitterion binds the metal center through O(carboxylate) atom. A co-ordinated water molecule completes hepta co-ordination in each case. Based on these data a structure of the type shown schematically in Figure 1 has been envisaged for complexes, 3 and 4 which is shown for the glycyl–glycine complex as a representative example.

Electronic spectra of compounds 3 and 4 in aqueous solution exhibited a weak intensity broad band at 240–250 nm (Table I) originating from co-ordinated peroxide. These bands are typical of LMCT transitions of diperoxo derivative of tungsten [4,8].

Thermal analysis

Thermogravimetric analysis data indicated that after the initial dehydration, the compounds 3 and 4

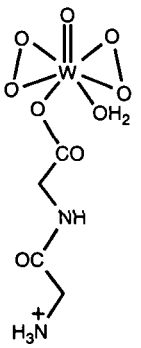

Figure 1. Proposed structure of the monomeric peroxotungstate compounds shown with $[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$ (3) as representative.

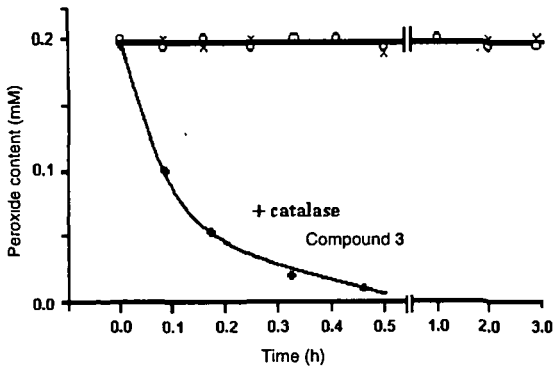

Figure 2. Stability of complexes at different pH values, effect of catalase on compound 3. (: Compound solution in distilled water (0.1 mM), pH of the solution 5.2. × : Solution of complexes in phosphate buffer (pH 7.0). : Effect of catalase. The test solution contained phosphate buffer (50 mM, pH 7.0) and the catalase (40 μg /mL) which was incubated at 30°C for 5 min. Compounds (0.1 mM) were then added to the reaction solution and aliquots were drawn at indicated time points and loss in peroxide content was determined.

undergo multistage decomposition. The complexes do not explode on heating. The TGA curve of compound **3** shows the first stage of decomposition occurring between room temperature and temperature of 110°C with the liberation of the outer sphere water molecules from the complex. The corresponding observed weight loss of 10.3% is in good agreement with the calculated value of 11.5% for the loss of three molecules of water of crystallization. The next decomposition stage is a two step one in the temperature range 110–240°C with a corresponding weight loss of 17.45% attributable to loss of co-ordinated water and peroxo groups from the complexes, which agrees well with the calculated value of 17.55%. The results thus provided conclusive evidence for the presence of co-ordinated, as well as lattice water in the compounds. Absence of peroxo group in the decomposition product, isolated at this stage, was confirmed from the infrared spectral analysis. The loss of peroxide is seen to be followed by another decomposition step occurring in the temperature range of 240–465°C. The corresponding weight loss of 27.17% for this decomposition agrees well with the value of 28.05% calculated for the loss of coordinated ligand (gly-gly) from the complex. The residue remaining at this stage was found to be an oxo-tungsten species as indicated by the IR spectra, which displayed the characteristic ((W=O) absorption and was devoid of bands attributable to peroxo and the dipeptide ligands of the original compound. Thermogravimetric analysis data of the compound thus provided further evidence in support of the composition and formula assigned to the compounds.

Stability of the complexes 1–4 in solution – their action with catalase

The investigations on the stability of the compounds in an aqueous solution of pH ca. 5, which is the natural pH attained by the solution of the compounds **3** and **4** in water, revealed that their peroxide content and position and intensity of their electronic spectral bands remained unaltered for over a period of 12 hours. Figure 2 shows that the compound **3**, used as a representative, is stable in solution of pH 5.5 as well as at pH 7.0. We further examined and ascertained their stability in solutions of pH values ranging from 3.6 to 8.0. Stability of these monomeric compounds was found to be comparable to those of dimeric amino acid or peptide containing pW analogues [26].

In the context of the present study which mainly focus on investigating some biochemically relevant properties of the pW complexes, it was considered imperative to examine the sensitivity of the pW complexes **1–4** towards catalase, the enzyme that catalyze the breakdown of H_2O_2 formed during oxidative processes in the intercellular peroxisomes. On incubation with catalase, each of the monomeric and dimeric pW compounds was found to be degraded gradually with the loss of peroxide. The effect of catalase on the complexe **3** is shown in Figure 2. Total peroxide loss from each of the pW compound solution of 0.1 mM concentration tested was recorded to be ca. 0.4, and ca. 0.2 mM for dinuclear and mononuclear compounds, respectively (Table II) indicating a ratio of 1:4 for peroxide: dinuclear complex (compounds **1** and **2**) and 1:2 for mononuclear pW compounds (**3** and **4**) which is in excellent agreement with the estimated peroxide content of the compounds. The extent and initial rate of degradation of the dimeric complexes **1** and **2** under the effect of catalase action (Figure 2) were

Table II. Catalase-dependent oxygen release from peroxotungsten compounds **1–4**.

Compounds	Conc(mM)	Loss of peroxide	
		μM /min.	Total(μM)
$Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$ (1)	0.1	21.3	39.2
$Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$ (2)	0.1	24.1	39.4
$[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$ (3)	0.1	6.8	19.1
$[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$ (4)	0.1	8.7	19.7

found to be comparable to that of $A_2[W_2O_3(O_2)_4(-cystine)].4H_2O$ (ca. 20 μM/min from a solution of 0.2 mM) [25] indicating their similarity with respect to number of peroxide and pattern of their co-ordination to the W(VI) center. Under the effect of catalase the rate of degradation of H_2O_2 with the release of oxygen was reported to be 430 μM/min [39] from a solution of 0.1 mM concentration and the reaction will be completed in less than 2 min. Thus the rate of H_2O_2 degradation is several fold higher than the rate of degradation observed for the title compounds under similar reaction conditions. It is thus evident that the synthesized pW complexes are at least 20 times weaker as substrates to catalase compared to H_2O_2, its natural substrate.

Oxidation of glutathione (GSH) by peroxo complexes 1–4

Oxidizing capacity of peroxometallates with respect to reduced GSH, were tested by the method of Ellman [30]. The monomeric as well as dinuclear peroxo tungsten compounds efficiently oxidized GSH, to GSSG. Amounts of GSH oxidized at two different concentrations of 5 and 10 μM of the compounds **1–4**, are presented in Figure 3. If all the four peroxo groups of the dimeric compounds (**1** and **2**) were active in oxidation, 8 moles of GSH to GSSG per mole of the compound should have been oxidized. Similarly, in view of the presence of two peroxo groups it is expected that compounds 3 and 4 would oxidize 4 moles GSH. However, the consistently observed stoichiometry of 4: 1 for GSH oxidized: dinuclear pW compounds (**1** or **2**) and 2:1 for GSH: mononuclear pW compounds (**3** or **4**) cause us to infer that only one of the peroxo groups of a diperoxotungsten, $WO(O_2)_2$ moiety of the monomeric compounds or two of the peroxo groups of the oxo-bridged tetraperoxo tungsten species, $[\{WO(O_2)_2\}_2O]$ of compounds **1** and **2** would be active in GSH oxidation.

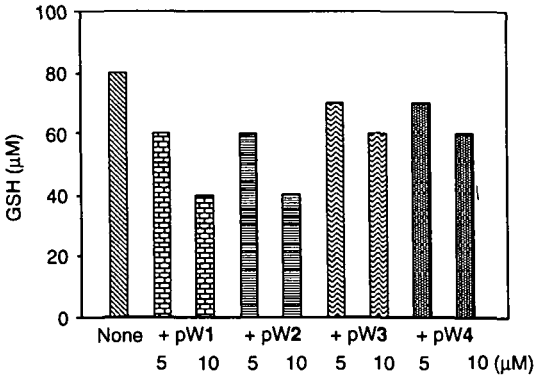

Figure 3. Oxidation of GSH by pW compounds 1- 4. The reaction mixture contained GSH (80μM) and phosphate buffer (50 mM, pH 7.0). A measured amount of the synthesized compound was added. Measurements were done by using two different concentrations of the compounds, 5.0 and 10.0 μM in triplicate under same assay conditions. Following incubation of 10 min, DTNB (160 μM) was added to the solution and the change in absorbance at 412 nm was determined in order to measure the GSH remaining in solution.

Effect of the compounds 1–4 on alkaline phosphatase activity

Alkaline phosphatase is a membrane-bound zinc metalloenzyme with a broad substrate specificity, which catalyzes the hydrolysis of organic phosphate monoesters possibly via an enzyme-phosphate intermediate. The maximum activity of the enzyme is observed at pH ≥ 8. Phosphotransferase activity and protein phosphatase activity are some of the other probable functions assigned to the enzyme.

In the present study, we have examined the effect of different concentrations of the newly synthesized peroxo-metal complexes upon ALP activity of rabbit intestine alkaline phophatase employing established enzyme assay system and p-NPP as substrate. The dose dependent effects of the mononuclear and dinuclear peroxo tungstate complexes **1–4** compared with the free ligands, W(VI), W(VI)- H_2O_2 systems, respectively are presented in Figure 4. We determined the half-maximal inhibitory concentration (IC_{50}) for each inhibitor, which gave rise to a 50% suppression of the original enzyme activity (Table III) in order to quantify the inhibitory potential of the molecules. The enzymatic rate ratios V_0/Vi where V_0 is the uninhibited rate and Vi is the rate of the enzymatic reaction inhibited by the complexes and other species were found to be directly proportional to the different compound concentrations (Figure 5).

Discussion

Tungsten has strong affinity for peroxide and the composition of peroxo-tungsten species has been known to be sensitive to pH [4,31,32]. In a solution of tungstate and excess H_2O_2 at a pH ≥ 5 diperoxotungstate species is readily formed, whereas the dimeric species, $[W_2O_3(O_2)_4]^{2-}$ predominates at pH ≤ 5 [4,31,32]. Thus in the present study, the strategically maintained pH of ca. 5 was found to be conducive for the successful synthesis of the molecular compounds 3 and 4. It is notable that maintenance of acidic pH of ca. 3 was one of the essential requirements for achieving syntheses of the amino acid or peptide contaning oxo-bridged tetraperoxotungstates **1** and **2** [25,26].

The dipeptide ligands contain several potential donor sites and as such, depending on the metal ion, pH and solution composition, can lead to a variety of co-ordination modes involving the terminal amino, carboxylate groups as well as amide linkage [33,40]. Thus the chosen ligands can act as mono, bi or

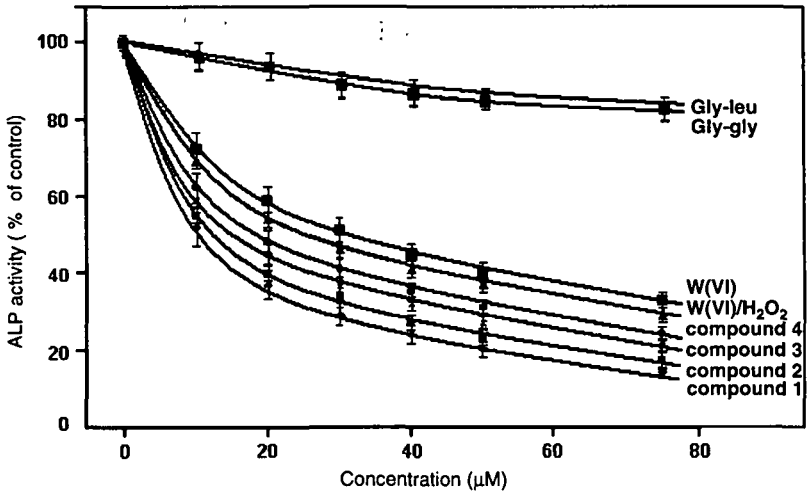

Figure 4. Effect of compounds 1 - 4, gly-gly, gly-leu, W (VI) and W (VI)/H_2O_2 species on activity of ALP from rabbit intestine. The ALP catalyzed rates of hydrolysis of *p*-NPP at pH 10.0 were determined at 30 °C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/mL), *p*-NPP (1 mM) in incubation buffer (25 mM glycine +2 mM $MgCl_2$, pH 10.0) in the absence or presence of stated concentrations of the inhibitors. Effects of the additions are represented as the percent values (rounded to integers) of control (Δp-NP = 0.96 μM/min). The data are presented as the means ± SE from three separate experiments.

tridentate species and can occur in complexes in either neutral zwitterionic form or anionic form. In the present case, the pH ca. 5 of the reaction medium is unlikely to allow deprotonation of the amide group of the peptide and thereby limits co-ordination to the metal centres through carboxylate group of the peptide zwitterions. Although the bi or tridentate co-ordination is more common in amino acid or peptide containing complexes however, monodentate co-ordination through a carboxylate group of the aminoacid or peptide zwitterion is not unprecedented [26,41–43]. The high stability of the compounds **1–4** in solution at acidic as well as physiological pH is likely to be due to the additional stability imparted by the ancillary ligands.

We have been particularly interested to investigate the interaction of the synthesized complexes with glutathione (GSH) in view of the literature report where it has been demonstrated that [10] the higher efficacy of the pertungstates and permolybdates as insulinomimetic agents is a consequence of their oxidizing activity relative to glutathione (GSH). Glutathione is the major non protein thiol in living cells, which plays the role of cellular reducing agents and antioxidant [44]. It was intriguing to note that, although the compounds **1–4** undergo degradation under the effect of catalase with total loss of peroxide, the oxidation activity of the pW compounds tested irrespective of being monomeric or dimeric, was limited to ca. 50% of that expected on the basis of the total number of peroxo groups present in these complexes. Similar observation was made earlier while investigating the GSH oxidizing ability of the complex species, $[W_2O_3(O_2)_4(cystine)]^{2-}$ [25]. The result of the investigation is consistent with the proposal implicating formation of a monoperoxo-W (VI) intermediate, which is inactive in GSH oxidation and is in accord with the earlier suggestions that for a peroxotungsten complex to be active in oxidation an oxo-diperoxo configuration may be a prerequisite [6]. Significantly, during one of our previous studies involving redox activity of the complexes **1** and **2** in oxidative bromination, only two of the peroxo groups out of 4 peroxides present per molecule of the complex were found to be involved in bromide oxidation [26]. However, there appears to be no plausible explanation as to why only half of the peroxide groups present per molecule of a pW complex should participate in oxidation.

The most noteworthy feature emerging out of our data derived from Vo/Vi relationships and IC_{50} values

Table III. Half-maximal inhibitory concentration (IC_{50}) values of the compounds **1–4** and other inhibitors against ALP.

Inhibitor	IC_{50} (μM)
$Na_2[W_2O_3(O_2)_4(gly\text{-}gly)_2].3H_2O$ (1)	10.29
$Na_2[W_2O_3(O_2)_4(gly\text{-}leu)_2].3H_2O$ (2)	12.67
$[WO(O_2)_2(gly\text{-}gly)(H_2O)].3H_2O$ (3)	15.84
$[WO(O_2)_2(gly\text{-}leu)(H_2O)].3H_2O$(4)	19.80
Tungstate	31.68
Tungstate/H_2O_2	25.34
Glycyl-glycine	–
Glycyl-leucine	–

Note: The ALP catalyzed rates of hydrolysis of *p*-NPP at pH 10.0 were determined at 30°C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/mL), p-NPP (1 mM) in incubation buffer (25 mM glycine +2 mM $MgCl_2$, pH 10.0) in the presence of stated concentrations of the inhibitors (Figure 4).

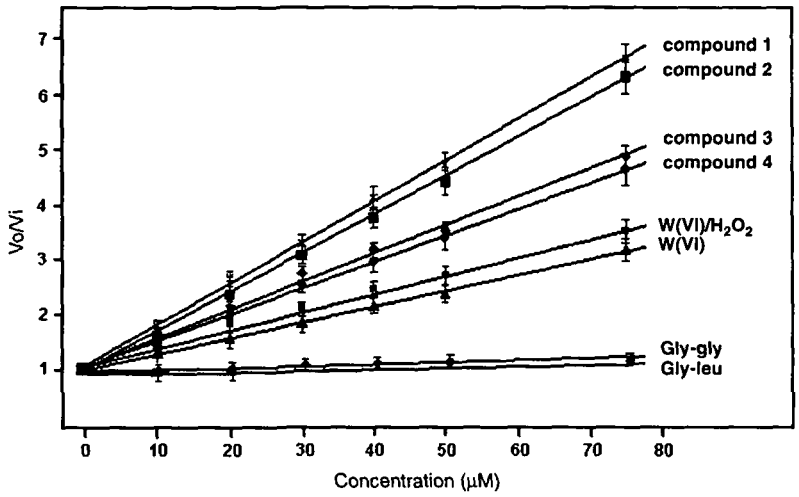

Figure 5. V_0/V_i ratios for the inhibition of compounds 1 - 4, gly-gly, gly-leu, W (VI) and W(VI)/H_2O_2 species in the alkaline phosphatase catalyzed hydrolysis of *p*-NPP. The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and p-NPP (1 mM). The reaction was started by adding ALP (3.3 μg/mL) to the reaction solution which was pre-incubated for 5 min and the rate of hydrolysis in the absence of the inhibitors, V_0, was obtained. The inhibited rates of hydrolysis, V_i, were determined as above and in the presence of stated concentrations of inhibitors. The values are expressed as means ± SE from three separate experiments.

is that although individually each of the tested species inhibited ALP activity to varying degrees, the peptide containing peroxotungsten complexes turned out to be the most effective inhibitors. Complexes with gly-gly as ancillary ligand are more potent inhibitors than the corresponding gly-leu containing analogue. Further, among the dimeric pW compounds **1** and **2** and previously tested compound $A_2[W_2O_3(O_2)_4$ (cystine)]. $4H_2O$ with an IC_{50} value of ca. 8.0 μM [25], the following trend in inhibitor potency has been noted: $[W_2O_3(O_2)_4(\text{cystine})]^{2-}$ > compound **1** > compound **2**. Previous studies indicated that phosphatases are, in general, inhibited by oxyanions such as vanadate [22–24], molybdate and tungstate [9,13]. Such inhibition has been attributed to the formation of pentaco-ordinated or hexaco-ordinated structures, which are described as phosphate analogues [9,13,22–24]. In the present study, it is notable that the inhibitor potency of the compounds tested, despite having hepta-coordinated metal center in each of them, is much higher than expected from equivalent concentrations of tungstate or peroxotungstate formed in solution. In this context it is also appropriate to recall the findings of Crans *et al.* that some six- or seven-coordinate vanadium or pV compounds inhibit phosphatases, however, five coordinated compounds are more potent inhibitors than the 6 or 7 co-ordinated ones [24]. The above observations demonstrate that structural analogy with the transition state or phosphate mimicry may not be the only factor due to which metal complexes may exert inhibitory effect on protein phosphtases. Although it is evident from our data that the inhibitor potencies of the compounds are sensitive to the nature of co-ligand environment, however, the possibility of the observed inhibition being caused by the amino acid or peptide co-ligand alone may be ruled out since the effect of individual ligands on ALP activity is practically negligible under the assay conditions used and H_2O_2 as such had no observable effect. In absence of direct evidence at this stage we are unable to discern definitive reasons for the effect of the title compounds on the phosphatase activity. On the basis of our observation made on of the pW complexes [25] in conjunction with the reports documenting the importance of redox properties of peroxo vanadium compounds in inhibition of protein phosphatases [22–24], we have previously proposed the oxidant properties of the title complexes as one of the possible factors responsible for making the compounds effective inhibitors of the phospho proteins [25]. Results of the present investigation lend further support to this proposal. Correlation has also been reported to exist between the oxidizing ability and insulin mimetic activity of peroxo compounds of tungsten and molybdenum [10].

Conclusions

The newly synthesized compounds represent a set of water soluble peroxo derivatives which contain species familiar to bioenvironment as ancillary ligand. The present experiments confirm that the pW compounds **1–4**, irrespective of being monomeric or dimeric, are potent inhibitors of ALP activity. An additional distinctive feature of the compounds, which may be of clinical relevance, is their high stability in solution at a wide range of pH values, particularly at acidic pH.

The compounds thus fulfill one of the criteria for metal complexes to be useful as therapeutic agent and provide future scope for testing such properties It is also noteworthy that the compounds are relatively resistant to degradation by the powerful enzyme catalase and utilise their peroxide groups only partially during interaction with GSH This may be relevant in the cellular milieu where H_2O_2 has little chance to survive abundant catalase and glutathione peroxidase

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