Studies on Peroxo Complexes of Vanadium(V). In Search of Novel Peroxovanadates of Biochemical Relevance

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To

The Department of Chemical Sciences
School of Science and Technology
Tezpur University
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India

2004


Dedicated to My
Beloved Parents

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List of Abbreviations

ADPV	alkali diperoxovanadate
asn	asparagine
BMOV	bis(maltolato)oxovanadium(IV)
bipy	2,2-bipyridine
dipic	pyridine-2,6-dicarboxylato
DPV	diperoxovanadate
dpot	1,3-diamino-2-propanol-tetraacetato
EDTA	ethylenediaminetetraacetic acid
ESI-MS	electrospray ionization mass spectrometry
gln	glutamine
gly-ala	glycyl-alanine
gly-gly	glycyl-glycine
gly-asn	glycyl- asparagine
gly-gly-gly	triglycine
LMCT	ligand to metal charge transfer
MPV	monoperoxovanadate
NAD	nucleotide adenine dinucleotide
NADH	nucleotide adenine dinucleotide reduced form
NTA	nitrilotriacetic acid
O-phen	o-phenanthroline
pic	picolinato
TMB	1,3,5-trimethoxybenzene
V-BrPO	vanadium bromoperoxidase
V-HPO	vanadium haloperoxidase

ABSTRACT

Studies on Peroxo Complexes of Vanadium(V). In Search of Novel Peroxovanadates of Biochemical Relevance

ABSTRACT

The present thesis deals with the results of studies involving synthesis, assessment of structure and reactivity of some novel peroxovanadium(V) complexes. The contents of the thesis have been distributed over eight chapters.

Chapter 1 presents a brief introduction pertaining to the work embodied in the thesis. The importance of and the interest in vanadium chemistry in general, and peroxo and heteroligand peroxovanadium(V) compounds in particular are highlighted. Attention has been drawn to the discoveries that dramatically enhanced the awareness of importance of vanadium and its compounds in biology. Also emphasized in this Chapter is the paucity of information concerning dinuclear peroxovanadates particularly with a peroxo-bridged moiety. This Chapter also projects the scope of work on the chosen aspects of vanadium chemistry.

Chapter 2 describes the details of the methods of 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.

Chapter 3 of the thesis presents an account of the reaction of alkali-metal diperoxovanadate (ADPV), a biologically relevant peroxovanadium compound, with vanadyl sulphate in the presence of EDTA which led to the synthesis of novel dinuclear heteroligand peroxovanadates(V) $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$, A=Na(**3.1**) and K(**3.2**).

Diperoxovanadate has been known to gain oxidant activity by complexing with vanadyl (V^{IV}) to form a highly reactive intermediate which could oxidize NADH and bromide or release oxygen in absence of any substrate. Certain ligating agents viz. EDTA, histidine, imidazole etc. were found to inhibit such redox processes. With an aim to ascertain the nature of the inhibitory species, it was considered worthwhile to investigate the reaction of DPV and VO^{2+} in presence of EDTA by attempting to isolate such species into solid state. The success of obtaining the solid complexes **3.1** and **3.2** depended on the following essential components : maintenance of the molar ratio of ADPV (A=Na or K) : VO^{2+}: EDTA at 1:0.75:1, order of addition of reactant, pH of 8.5, maintenance of required reaction time as well as temperature at ≤4°C. Compounds were precipitated with ethanol. The compounds were characterized by elemental analysis, magnetic susceptibility and spectral studies. Important structural features of the complex species include the presence of EDTA bridge and co-ordinated sulphate. One of the vanadium(V) of the dinuclear species contains a bidentate peroxide, whereas the other vanadium center is bonded to a unidentate sulphate and water leading to hepta co-ordination around each vanadium(V).

The electronic spectra of the complexes **3.1** and **3.2** displayed a broad LMCT band at 390-400 nm owing to peroxo (LMCT) transition. The complete loss of the 780 nm band in the spectra of the complexes characteristic of $VOSO_4$ indicated oxidative

loss of V^{IV} during complex formation. Occurrence of vanadium in the complexes in its +5 oxidation state was further evident from their being diamagnetic and ESR silent.

Oxygen release reactions, molar conductance measurements and electronic spectral studies revealed that the compounds were stable in solution. No oxygen was released from the solution of the complexes in phosphate buffer (pH 7.0) even on treatment with catalase and incubating at 30°C for upto 30 min, confirming the resistance of the compounds to the enzyme. Compounds were unable to oxidize NADH and were inactive in bromination of phenol red into its 592 nm absorbing brominated product, bromophenol blue, at physiological pH. It is proposed that this dinuclear complex species corresponds to the complex formed in solution responsible for EDTA induced inhibition of DPV-V^{IV} mediated redox processes. These complexes are probably the first known peroxovanadate compounds containing co-ordinated sulphate.

Reported in **Chapter 4** are the first synthesis, structural assessment of dinuclear complexes, $Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$ (**4.1**) and $Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$ (**4.2**), and studies on stability and reactivity of these complexes.

The desired syntheses could be successfully achieved by adopting a synthetic strategy almost similar to the one used for the synthesis of $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$. The reaction of Na-DPV with $VOSO_4$ in presence of nitrilotriacetic acid (NTA) or glycyl-glycine were carried out maintaining the molar ratio of DPV : VO^{2+}: NTA as 1:0.75:1.5 and of DPV : VO^{2+}: gly-gly as 1: 0.5: 1.5. Suitable pH for the synthesis of compounds **4.1** and **4.2** were found to be *c.*7 and *c.*9, respectively.

The compounds were observed to be diamagnetic and ESR silent in conformity with the occurrence of vanadium in its +5 oxidation state in each of them. The presence of terminally bound peroxo group, terminal as well as bridging oxo groups and unidentate sulfate in the dinuclear complexes **4.1** and **4.2** was evident from their IR spectral pattern. IR spactra also indicated the co-ordination of the NTA to V(V) centre as a tridentate ligand in complex **4.1** and the occurrence of gly-gly bonded in a tridentate fashion through its O(carboxylate), O(amide) and $-NH_2$ group in complex **4.2**.

Results of oxygen release reactions, molar conductance measurements and electronic spectral studies confirmed the stability of complexes in solution and their resistance to catalase action. These compounds were unable to oxidize NADH and bromide. The reaction between DPV and VO^{2+} may thus serve as a paradigm for the synthesis of stable dinuclear heteroligand peroxovanadates when carried out in presence of suitable organic ligands.

Chapter 5 describes the synthesis and structural assessment of dinuclear peptide peroxo-vanadium(V) complexes, $[V_2O_2(O_2)_3(dipeptide)_3].H_2O$ [dipeptide = glycyl-glycine(**5.1**), glycyl-alanine(**5.2**), or glycyl-asparagine(**5.3**)] and $[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2].H_2O$ (**5.4**) with the distinctive features of having a $\mu\text{-}O_2^{2-}$ group and peptides as heteroligands. Also reported in this Chapter are the results of studies of nature and stability of the complexes in solution and their interaction with the enzyme catalase.

The compounds have been synthesized from the reaction of V_2O_5 with 30% H_2O_2 and the respective peptide. The pH value of *c.*2 attained spontaneously during the reaction was not raised. The molár ratio of V : peptide : H_2O_2 was maintained as

1:1.5:48.3. The reactions were carried out at an ice-bath temperature (≤4°C) and the precipitation of the complexes were brought about by the addition of ethanol. The compounds were characterized by elemental analysis and spectral studies including IR, LR and electronic spectroscopy. IR and Raman spectral data suggest the presence of two different types of peroxo groups in each of the complexes viz., terminal chelated and bridging type. Peptide ligands occur as zwitterion in each of the compounds. The compounds were diamagnetic in nature in conformity with the presence of vanadium(V).

The compounds were observed to undergo rapid degradation in aqueous solution with partial loss of peroxide accompanied by release of oxygen. The ^{51}V-NMR spectra of such solutions showed diperoxovanadate and decavanadate as the products. Additional oxygen was released on treating these solutions with catalase as expected of residual diperoxovanadate.

Chapter 6 deals with the results of investigations on the reactivity of the complexes, $[V_2O_2(O_2)_3(dipeptide)_3].H_2O$ [dipeptide = glycyl-glycine(**5.1**), glycyl-alanine(**5.2**), or glycyl-asparagine(**5.3**)] and $[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2].H_2O$ (**5.4**) in oxidative bromination, NADH oxidation and its effect on the activity of the enzyme glucose oxidase.

The peroxobridged complexes were highly effective in generating bromination competent intermediate at physiological pH. The bromination activity of the complexes **5.1-5.4** was tested by adding weighed amount of the solid compound to the standard reaction mixture of bromide in phosphate buffer with phenol red as the trap for oxidized bromine. The color of the solution instantly turned blue and the spectrum showed a

jump in absorbance at $\lambda_{max} \approx 592$nm characteristic of the product bromophenol blue. After the instant activity, a slow increase in A_{592} indicated a secondary rate of bromination. Based on spectroscopic studies a mechanistic pathway involving a sequence of mono, di, μ- and Br-peroxovanadate 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.

The compounds were, however, inactive in oxidation of NADH and also were unable to inactivate the enzyme glucose oxidase. This demonstration of peroxide-bridged divanadate as a powerful, selective oxidant of bromide, active at physiological pH, should make it a possible candidate of mimic in the action of vanadium in bromoperoxidase proteins.

In **Chapter 7** the synthesis and characterization of dinuclear and mononuclear peroxovanadates containing asparagine or glutamine as co-ligands are described. Results of a comparative study of the stability and redox properties of the two types of complexes are also reported herein.

Dinuclear complexes $[V_2O_2(O_2)_3(asn)_3]H_2O$ (**7.1**) and $[V_2O_2(O_2)_3(gln)_3].H_2O$ (**7.2**) have been synthesized from the reaction of H_2O_2 with V_2O_5 and the respective amino acid ligand, asparagine or glutamine, at pH *c.*2. Similar reactions conducted at pH *c.*5 afforded the monomeric complexes $Na[VO(O_2)_2(asn)].H_2O$ (**7.3**) and $Na[VO(O_2)_2(gln)].H_2O$ (**7.8**).

From the elemental analysis data, the ratio of V : O_2^{2-} and V : amino acid was ascertained to be 2:3 for complexes **7.1** and **7.2** suggesting a dimeric nature of the complexes. IR spectral pattern of the complexes **7.1** and **7.2** indicated the presence of

terminally bonded and bridging peroxo groups in these complexes and occurrence of the amino acids in their zwitterionic form co-ordinated through carboxylate group. The monomeric complexes **7.3** and **7.4** contain terminal chelated peroxides and an amino acid ligand binding the V(V) centre as a bidentate ligand through O(carboxylate) atoms.

These dinuclear and mononuclear peroxovanadate(V) complexes were not only different in their composition and spectral pattern, but also showed significant differences in their nature, stability and redox activity in solution. The complexes **7.1** and **7.2** rapidly degraded in solution with release of O_2 and formation of diperoxovanadate and decavanadate as shown by ^{51}V-NMR spectra whereas, complexes **7.3** and **7.4** remained stable in solution for over 24 hours. The extent and the rate of oxygen released from the two types of complexes under the effect of catalase action further evidenced the differences in their V : O_2^{2-} content and mode of peroxide bonding in these species.

Peroxo-bridged divanadates, **7.1** and **7.2** proved to be powerful oxidant of bromide at physiological pH, which could also mediate bromination of organic substrates in aqueous-organic media. Compounds **7.3** and **7.4** were found to be inactive in bromination under analogous condition. The results provide further evidence for the 'VOOV' group to be the active bromide oxidant at physiological pH.

In **Chapter 8**, 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 results of studies described in **Chapters 3**, **5**, and **6** have been published, while the results incorporated in **Chapter 7** has been accepted for publication. Work mentioned in **Chapter 4** is now under communication.

Chapter 3

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Chapters 5 and 6

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Chapter 7

Polyhedron (accepted for publication).

Introduction

1.1 VANADIUM – HISTORICAL PERSPECTIVE AND OCCURRENCE

Vanadium, a group V transition element with outer electronic configuration $3d^3 4s^2$, is ubiquitous in nature[1]. The element was first discovered in 1801 by a mineralogist Andres Manuel del Rio in a brown lead mineral from Mexico[2,3]. 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[2]. However, vanadium was rediscovered[4] almost 30 years later 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[5-7] is approx. 0.02%. In ocean it is the second most abundant transition element (50nM)[8]. 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[3,5]. It is generally the most abundant trace metal in crude oils and in shales[3,9] where it is present in the form of organic complexes.

Vanadium compounds are also found in traces in biosphere[6,10]. It is normally present at very low concentrations in virtually all cells in plants and animals[11]. In mammals it is an ultratrace element which is widely distributed in tissues[12]. Several ascidians accumulate high concentrations of the element in lower oxidation states in their blood cells[9]. However, the nature of vanadium species and its role in these bio-systems remain unclear[13]. Vanadium is found naturally associated with two types of

enzymes, haloperoxidases found in marine organisms[14] and certain nitrogenases of nitrogen-fixing bacteria (*Azotobacter*)[15]. Some accessory foods such as black pepper, tea leaf, cocoa powder and some mushrooms contain relatively high amounts of vanadium.

Besides its utility as an oxidation catalyst in industrial processes[16,17], vanadium is now considered essential in trace quantities[18-20], has known therapeutic applications[6,7,11,21-37], and is toxic in excess[37-41].

1.2 THE GROWING IMPORTANCE OF VANADIUM IN BIOLOGY

Several major discoveries on biological effects of vanadium[9,11-15 42-46] 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[47] 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[48,49]. These reports marked the resurgence of interest in finding anti-diabetic vanadium compounds with low toxicity[6,7,11,26-29,32-35,50-53], and identification of peroxovanadates as possible active compounds that activate directly the cascade of enzymes that normally follows activation of insulin-receptor[54]. 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[55]. 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[22]. Finally, with the discoveries of proteins containing bound vanadium as a native constituent and essential for the activity of a bromoperoxidase[14] in a marine alga, in the year 1983, and of nitrogenase in *Azotobacter*[15] the biological role of vanadium has been firmly established.

Table 1.1. *Resurgence of interest in biological actions of vanadium*[46]

Reaction/Parameter	Vanadium	Effect/Locale	Reference
Na,K-ATPase	vanadate	inhibition	47
Insulin-mimic	vanadate	blood glucose$^-$	49
Insulin-mimic	vanadyl	blood glucose$^-$	48
Noradrenaline-mimic	vanadate	arterial contraction	61
NADH-V reductase	vanadate	plasma membrane	62
NADH-O_2 oxidation	polyvanadate	plasma membrane	55
Bromoperoxidase	vanadate	marine alga	14
Nitrogenase mutant	vanadate	*A. vinelandii*	15

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[46,56]. A metavanadate containing tonic (neogadine) is available in the market in India. Most food materials used for human consumption contain vanadium in concentrations[20] below 0.1 μg/g. Dietary supplement of vanadate increases its tissue content which is stored in a non-toxic form[57]. However, pharmacological potential of vanadium has been systematically explored only in the last decade or so[22]. 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[50]. Yet, they have limited clinical usefulness so far due to several factors including toxicity of the metal[38].

Concomitant with renewed biological interest there has been an increasing interest in elucidating the chemistry of vanadium complexes as its co-ordination chemistry plays a central role in the interaction with biomolecules[8-10] as well as in catalytic oxidations[58-60].

1.3 SELECTED ASPECTS OF VANADIUM CO-ORDINATION CHEMISTRY

The co-ordination chemistry of vanadium has achieved a special status in the last decade[63] owing mainly to the model character of many vanadium complexes for the biological function of vanadium[35,59,60,64-70], the use of oxo V complexes in oxidation and oxo transfer catalysis[60,71-73], and potential medicinal applications[7,8,11,32-35,74]. In order to understand how vanadium might function in relatively complex biomolecules it is incumbent on us to understand its basic co-ordination chemistry with simpler ligands. 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[63]. Vanadium(IV) and vanadium(V)

oxidation states are more common and are stable under ordinary conditions[63]. Vanadium studies remained in low profile due to its exceptionally complicated chemistry in solution[10,75]. 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[76].

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[77]. 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. The V(IV) is a radical and can be easily detected by the characteristic 8-banded ESR spectrum originating from hyperfine interaction of ^{51}V nucleus (I = 7/2). V(V) is EPR silent due to its d^0 state. Vibrational spectroscopy plays an important role in the study of oxo-vanadium compounds. The most characteristic feature of the vibrational spectra of oxo-vanadium complexes is the occurrence of very strong and sharp bands at 980 ± 50 cm^{-1}. Such bands are assigned to the V=O stretching frequencies[78,79] and as expected it lies near the upper frequency limit for those complexes which are known, from X-Ray work, to have the shortest V-O bonds[80].

Vanadyl interacts readily with carbonate[10,30], phosphates[10,81,82], pyridine, imidazole and other amine bases[10,81,82] and form different complexes. Hydrocarboxylic acid, phosphocarboxylate, nucleosides, nucleotides, catecols[10,81-84] etc. which contain more than one functionality form strong complexes with vanadyl cation[10]. These reactions are of physiological interest. Interaction of vanadyl with cysteine, cystene[27], picolinic acid[29], N, N-ethylenediamine diacetic acid[85] 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[11,74]

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[86]. 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[10]. Vanadium is stereochemically flexible with coordination geometries ranging from tetrahedral and octahedral to trigonal pyramidal and pentagonal bipyramidal being thermodynamically plausible[87]. 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[86,88]. Commonly used organic buffers and EDTA form complexes with vanadium compounds[86].

One of the most interesting aspects of vanadium chemistry, which has also engaged the attention of several groups of contemporary researchers, is its peroxo chemistry[35,54,59,80,89-94]. Peroxo-transition metal complexes in general have received continued attention over several years because of their important roles in biological processes[95-97] and in catalytic oxidations[59, 97-106].

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[95,96,107-109] as well as industrial chemists interested in developing homogeneous analogues to heterogeneous metal-catalysed oxidation reactions[58-60,98-106]. 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[59,110-114].

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[115]. 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 1.2.

Table 1.2. *Physical data for dioxygen species*[97]

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[115], 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[96], 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[6,59,80,91,96,97,112] (F^-, Cl^-, NH_3, SO_4^{2-}, $C_2O_4^{2-}$, CCO_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 $Op\pi^*$ orbitals of the peroxide ion[96]. 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[95,96,109].

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 semiimperical computations[114] (*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.

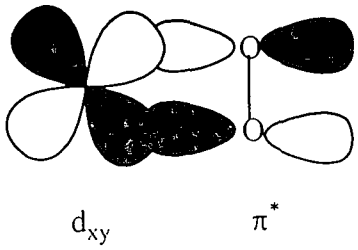


Fig. 1.1 Interaction of metal d_{xy} orbital with peroxo π^* orbital. Formation of metal-peroxo bond in peroxo metallates[114].

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[115] can be represented as shown in *Fig. 1.2.*

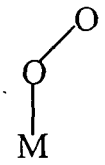
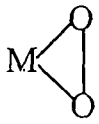
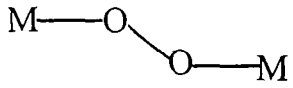
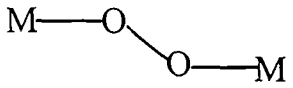
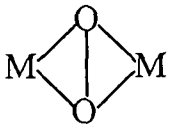
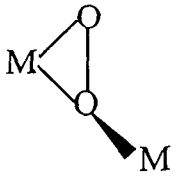
Structural type	Structural designation	Vaska classification
	η^1 dioxygen	Type a (superoxo)
	η^2 dioxygen	Type IIa (peroxo)
	η^1 : η^1 dioxygen	Type Ib (superoxo)
	η^1 : η^1 dioxygen	Type IIb (peroxo)
	η^2 : η^2 dioxygen	—
	η^1 : η^2 dioxygen	—

Fig. 1.2 Structural classification of metal-dioxygen complexes[115].

The bridging peroxo could vary from cis-planar and trans-planar to trans-nonplanar configuration. An unusual symmetrical double bridging was also found[116,117].

Deviations from the ideal symmetry are also observed very often[97,118,119]. 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 arrangement is most common for transition metal complexes, usually with two co-ordinated peroxo groups in cis position.

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[110] and many peroxo transition-metal compounds have been isolated in the solid state[59,96,110,112]. Peroxo-metal complexes besides having an intrinsic interest of their own[59,93,112] constitute an important class of reactive intermediates in catalytic oxidations[99,100,120-125] and are involved as potential oxygen donors in the oxygen transfer reactions to organic substrates including hydrocarbons[93,100,101,120-126]. 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[127,128].

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[110] 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[96,129-131]. The metals, Sc, Ti, V, Cr, Y, Zr, Nb, Mo, La, Hf, Ta, W[96] and U[132] form stable heteroligand peroxo

complexes. The biochemical significance of peroxo metal complexes has been emphasized in literature[95,96]. The reactivity of peroxides[100,101,114,133-135] 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.

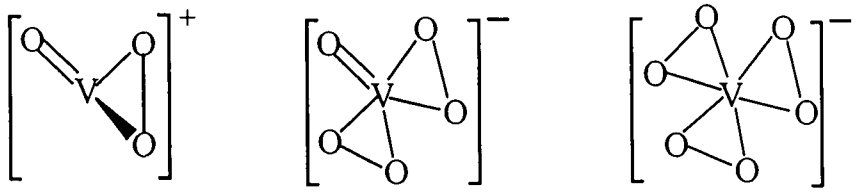
1.5 PEROXO COMPOUNDS OF VANADIUM – CHEMISTRY AND IMPORTANCE

Peroxo-vanadium chemistry has increasingly become the center of research attention. Knowledge regarding the active involvement of peroxovanadium compounds in haloperoxidases[68,136,137], their enzyme inhibitory[136], antineoplastic[21,23], and insulino-mimetic properties[6,7,11,25-29,32-35,50-53] as well as their potent catalytic properties in the oxidation of organic and inorganic substrates[59,100-102,120-125,137-139] have intensified interest in these complexes.

Vanadium-hydrogen peroxide system appears 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[93,110]. The composition of peroxovanadium species formed in aqueous solution is sensitive to various factors viz., vanadium and hydrogen peroxide concentration, pH, ionic strength, and reaction temperature.

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[93,110,140,141]. 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[93,110,140,141]. At higher peroxide and vanadium ratio and pH > 8.0 triperoxo[142] and tetraperoxo species

dominate[110]. Most species have pH dependent ^{51}V-NMR chemical shifts arising from protonation and deprotonation reactions and were characterized by ^{51}V-NMR spectroscopy[140,141,143-145] (Table 1.3). Study of ^{51}V-NMR spectra of these compounds proved to be invaluable tool in identification of vanadium(V) reaction intermediates and compounds formed.



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[93,110,140-142] 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[6,59,80,96]. Thus, depending on the pH and reaction conditions monoperoxo, diperoxo or triperoxo complexes may be

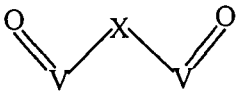
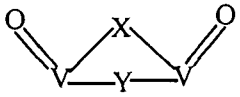
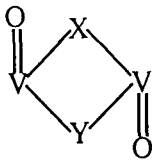
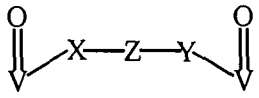
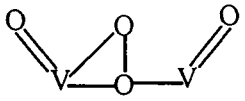
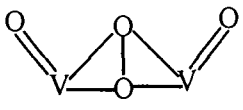
Table 1.3. *Species, formulae, ^{51}V-NMR peaks (ppm) of some vanadium compounds*[46]

No. of peroxo groups	Species	Chemical shift (δ) in ^{51}V-NMR peaks (ppm)		
		Howarth & Hunt (1979)	Harrison & Howarth (1985)	Jaswal & Tracey (1991)
Nil	OVO^{+}		-546	
	VO_4^{3-}	-545	-541	
	HVO_4^{2-} (V_1)	-534	-539	-536
	$H_2VO_4^{-}$ (V_1)	-574	-564	-566
	$HO(VO_3)_2^{3-}$ (V_2)	-562		-574
	V_4 cyclic			-578
	V_4 linear or V_5			-582
One	$VO(O_2)^{+}$	-543	-549	
	$VO(OH)_2(O_2)^{-}$	-621	-602	
	$VO_2(OH)(O_2)^{-}$	-623	-628	-625
	$[VO_2(O_2)]_2O^{4-}$ (dimer)		-636	
Two	$VO_2(O_2)_2^{3-}$	-760	-769	
	$V(OH)_2(O_2)_2^{-}$	-696	-700	-686
	$VO(OH)(O_2)_2^{2-}$	-767	-771	-765
	$[VO(O_2)_2]_2OH^{3-}$ (dimer)	-757	-767	-758
	$[V(OH)_2(O_2)_2]_2O$ (dimer)	-650		
Three	$VO_2(O_2)_3^{3-}$	-845	-830	
	$VOH(O_2)_3^{2-}$	-733	-737	

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[6]. A large number of peroxvanadium and oxodiperoxovanadium(V) complexes in diverse ligand environment have been structurally characterized and reported in recent years[6,59,80,91,146,147]. 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[6,59,80,91,146,147].

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[91] (Table 1.4). Examples of structurally characterized dinuclear peroxovanadates are listed[91] in Table 1.5. 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[80]. Dinuclear peroxovanadate intermediates possessing a μ-peroxo bridge have been implicated in certain biochemical processes[68,148-151]. However, only a few reports regarding chemistry of such species of vanadium in solid state are available[116,152-154]. The synthesis of peroxo-bridged vanadats and studies on their various properties, therefore, appear to be worthwhile area of investigation.

Table 1.4. *Bridge configurations found in dinuclear vanadium(V) peroxo complexes*[91]

Type	Structure	Type of Bridging
A		μ—X
B		μ—X, μ—Y, nonplanar bridge
C		μ—X, μ —Y, planar bridge
D		μ—X—Y—Z
E		$\mu-\eta^1:\eta^2O_2$
F		$\mu-\eta^2:\eta^2O_2$

X and Y are donor and Z are other atoms of ligand.

Table 1.5. *Structurally characterized dinuclear peroxovanadates*[91]

Dinuclear	Bridge	CN	Ligand(s)	Ref.
$[V_2O_2(O_2)_2)L_r]$ r = 4, 5	C,-	7	citrato	59
	C,-	7	malato	155
	B, A	7	L-tartrato, H_2O	91
	B, A	7	D-tartrato, H_2O	91
	C,-	6	glycolato	91
	C,-	6	DL-lactato	91
	C,-	6	DL-mandelato	91
	A, D	7	dpot	156
$[V_2O_2(O_2)_3)L_p]$ p = 3, 4	A, F	7	3F	59
$[V_2O_2(O_2)_4)L]$	E, -	6	H_2O (3)	59
	A, E	6-7	O	59
	A, E	6-7	OH (2)	59
	D, E	7	PO_4	157

A, B, C, D, E and F denote the type of bridging described in Table 1.4.

Modern spectroscopic tools are highly informative in the study of vanadium peroxo complexes. Peroxovanadates species formed in aqueous solution have been studied by several techniques including ^{51}V-NMR spectroscopy[135,140,141,143-145], ^{17}O-NMR spectroscopy[114,144], Raman spectroscopy[145] and by electrospray ionization mass spectrometry (ESI-MS)[138,158,159]. Moreover, structures of vanadium peroxo derivatives are also being theoretically investigated[138,159].

Infrared spectra are essential for the characterization of peroxovanadate compounds. Coordination of peroxide in a side-on bidentate fashion creates a local C_{2v} symmetry which has three IR active modes[160], symmetric O-O stretching, symmetric metal-peroxo stretching, and antisymmetric metal-peroxo stretching which occur at approximately 880, 600 and 500 cm^{-1} respectively[59]. 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. In the UV-Vis spectra of monoperoxo and diperoxo complexes a distinct difference is found. The ligand to metal charge transfer bands appears at around λ_{max} ~ 320 nm in diperoxo complexes whereas monoperoxo complexes absorb at a much lower energy with the λ_{max} ~ 420 nm[80].

In addition to the increasing evidence of the biochemical importance of vanadium, the efficiency of peroxovanadium complexes in oxidizing certain organic[93,100-102,161] and inorganic substrates[59,162,163] are notable. Peroxovanadium species usually react with organic substrates by generating O_2 and some reduced form of vanadium. Various synthetic approaches have been developed for the oxidations of alkenes and allylic alcohols to corresponding epoxides[59,100,101,138,164], primary and secondary alcohols to the aldehydes and ketones[59,138,165,166], aldehydes to esters[167,168], sulfides to sulfoxides and sulfones[59,102,104,138] as well as, hydroxylations of alkanes and arenes[59,100,138,164,169] (*Fig. 1.4*). The catalytic applications of peroxovanadates take advantage of the increased oxidation rate of peroxovanadium complex, which after

formation reacts[170]. Owing to the biological significance of the peroxo-vanadate mediated oxidations, most of the recent studies have been conducted in water[138,161,171,172]. However, peroxovanadate compounds are also efficient oxidizing agents in less polar organic solvents[101,164,173]. Recent developments have been utilizing biphasic, phase transfer systems as well[174,175].

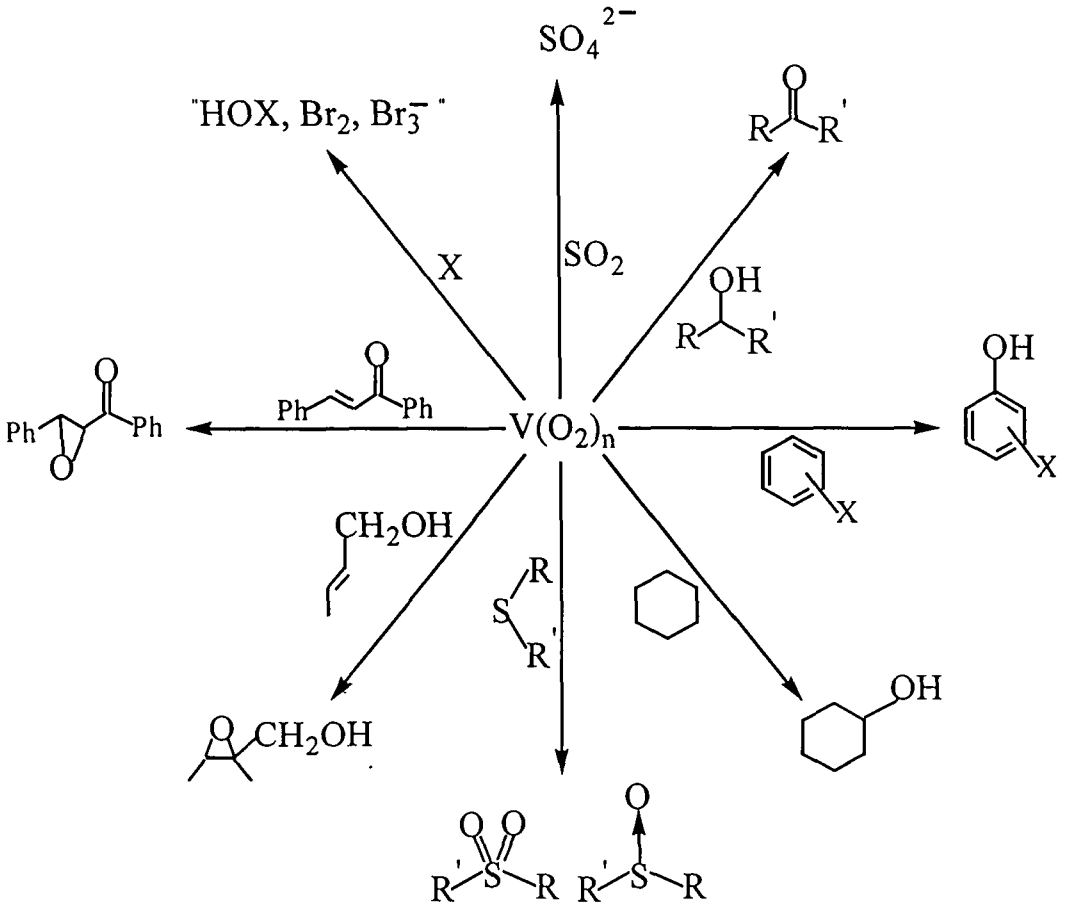


Fig. 1.4 Reactivity of vanadium peroxides with inorganic and organic substrates[138]

The stoichiometric or catalytic oxidations accomplished by peroxovanadates are usually carried out under mild conditions with good selectivity and chemical yields[59]. Mimoun el al. synthesized a series of vanadium complexes of tridentate Schiff base ligand which exhibited clean stereoselective epoxidation[101]. Some peroxovanadium complexes like $VO(OOtBu)(dipic)(H_2O)$, $VO(OOtCMe_2Ph))(dipic)(H_2O)$ etc. oxidize alkenes to a mixtures of products, primary allylic alcohols, ketones and aldehydes with a small amount of epoxides without selectivity[173]. One of the most striking features of the V(V) peroxo complexes is their ability to oxidize arenes, alkanes and alcohols[59,100,138,164-166,169]. Complex $VO(O_2)(OR)$ catalyze the oxidation of 2-propanol to acétone stoichiometrically with respect to H_2O_2 consumption[59,165]. The heteroligand peroxo complexes $VO(O_2)(O\text{-}N)LL'$ (O-N = pyrazine-2-carboxylate and $L,L' = H_2O$, or a basic ligand, e.g., pyridine N-oxide) could efficiently transform olefins to epoxides as well as hydroxylated aromatic hydrocarbons to phenol and alkanes to alcohols and ketones[164].

The nature of the coordinating ligand and the solvent system are very important factors on which the oxidative reactivity of peroxovanadate complexes depends[176]. 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 one electron acceptor and as oxidant[135]. The activity of peroxovanadium complexes as catalysts have been fine-tuned with ligands and various correlations have been made involving the electronics and other properties of the ligand[113,135,170-172,176]. The mechanism of oxidation reactions mediated by peroxovanadates as electrophilic or radical oxidants have been studied extensively[59,101,164,172].

Besides the oxidations of organic substrates, peroxovanadium complexes are also able to oxidize various inorganic substrates including of sulfur dioxide[162], thiocyanate[163] and halides[59,137]. The oxidation of halides with peroxovanadates is of particular interest as such a process is actually a chemical model of the activity of vanadium-dependent haloperoxidases[59,68].

Haloperoxidases are enzymes that catalyse 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[14,42,59,63,68,177].

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

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[14,42,59,63,68,177]. 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[177] 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[59,178]. The dispropotionation 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[59,102,104,138].

Crystal structures of some haloperoxidase proteins *Curvularis inequalis*[179], *Ascophyllum nodosum*[180], *Corallina officinalis*[181] 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[182]. 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 removel of vanadate[44] 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-BrPO have been extremely useful in helping to unravel details of the structure and mechanism of activity of the enzyme[64]. Selected structural models[64] of the enzyme are shown in *Fig 1.5*. Versatility with respect to coordination number, geometry and coordination functions is evident in the compounds. The common feature in these compounds is that their coordination sphere is dominated by oxygen functions, one or two of which are oxo groups. Compounds **3** and **4** are functional mimics which catalyze bromination of organic substrate under mildly acidic conditions[64].

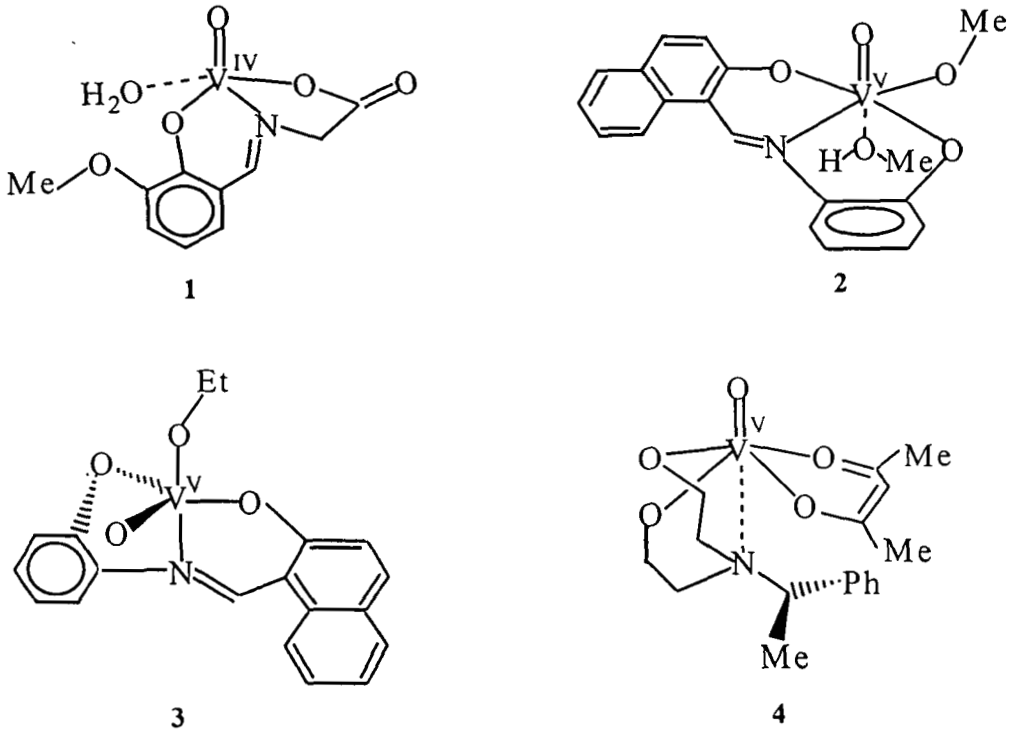


Fig. 1.5 Structural models for the vanadium site in peroxidases[64]. A dashed line (in **1**, **2** and **4**) represents a weak bond. The supporting ligands are Schiff bases (**1-3**) or ethanolamine (**4**)

In order to get a better understanding of the mechanism of action of the enzyme and to determine the role of vanadium many functional mimics for V-BrPO were developed[59,64-70,137]. The biomimetic functional models reported in the literature, some of which are discussed in Chapter 6 of this thesis, are mostly based on monoperoxo vanadium[65] or on triperoxo divanadium species[46,68,69,148,149]. Aqueous solution of cis-dioxovanadium(V) (VO_2^+) in acidic medium[69], a V_2O_5 and H_2O_2 system[70] as well as , KBr in excess H_2O_2 in presence vanadyl sulfate in phosphate buffer[178] (pH 6) were all

found to be effective in bromination of organic substrates and were studied in detail as functional mimic of the enzyme.

In recent years several peroxo-vanadium compounds and vanadium complexes, of multidentate ligands containing O and N donor sites were examined for catalysis of bromide oxidation with an aim to address the role of the protein environment around the active site[63-65,183]. Most of these model complexes were however, found to be catalytically active in acid medium whereas natural V-BrPO is most efficient[59] at pH 5.5-7. Thus it is evident that despite the progress made in gaining an insight into the various aspects of activity of V-BrPO, the exact mechanistic details of the enzyme function is yet to be fully understood and hence is still a subject of study.

Concomitant with the biochemical interest on the activity of V-BrPO there have been efforts to develop catalytic protocols with synthetic V-BrPO mimics[105,184,185]. 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[184]. The search for functional biomimics of the haloperoxidases, particularly to elucidate the mechanism of halogenation of organic substrates led to the discovery of several transition metal complexes as effective catalysts of the oxidation of halide by hydrogen peroxide[59,64,65,67-70] which indeed is an important development in this area. It would be useful to develop peroxovanadium compounds with definite potential for application as safer alternative synthetic catalyst for organic bromination reactions.

Other very important aspects of vanadium peroxide systems of contemporary interest are their antineoplastic[21,23] and insulin mimetic[6,7,11,25-29,32-35,50-53] effects. Djordjevic et al. tested a range of heteroligand peroxovanadate compounds for their

antitumour activity and observed that such activity was dependent on the nature of the hetero-ligand and the cations present[23]. According to very recent reports, diperoxovanadate complexes were found to be effective as drug for treatment of infectious deseases, in immune disorders or infections caused by viruses such as HIV virus, and in enhancing antimicrobial efficacy of drugs[186].

Vanadate and peroxide have been known to act synergistically to mimic insulin activity. Peroxovanadates are far more potent in facilitating the metabolic effects of insulin than vanadate[187]. 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.[6]. Mechanism by which peroxovanadates mimic insulin is not fully understood. A good correlation exists between the PTPase inhibitory abilities of peroxovanadate complexes, their abilities to promote activation of insulin receptor[188], and their *in vivo* insulin mimetic activities. It has been proposed that peroxovanadium complexes bind to the active site of PTPase because they are quite similar to phosphate ester and inhibit PTPase by irreversibly oxidizing the cystein residue in the active site of the enzyme[6]. 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[59]. A large number of heteroligand peroxovanadate complexes have been tested for possible insulin like activities which revealed a range of stabilities towards decomposition in aqueous solution, depending on the nature of the heteroligand[189].

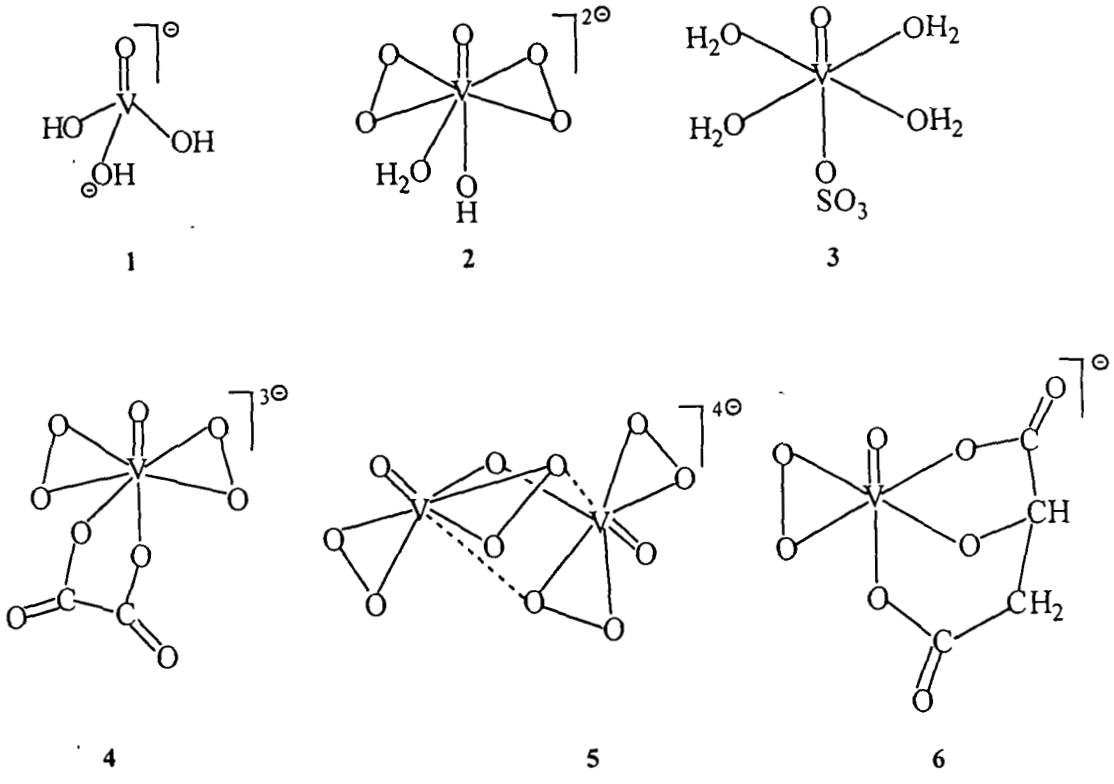


Fig. 1.6 Vanadium compounds of therapeutic importance. These compounds have already been proved to be active in animal tests[8].

However, most of these compounds are hydrolytically unstable and end with radical formation when subjected to redox processes[52,190] 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.

1.6 REASEARCH OBJECTIVES

It may be inferred from the above non-exhaustive discussion, that the chemistry of peroxovanadates(V) in general embraces a fascinating, rewarding and worthwhile area of investigation. Also it is evident that the synthesis of well defined peroxovanadate(V) complexes and assessment of their structure, stability and redox properties are above all prerequisites following which other aspects can be developed.

Accordingly, in line with the scope highlighted above, the research described in this thesis has mainly been focused on synthesis and characterization of novel peroxovanadium compounds, and investigating their stability and redox activity in a variety of oxidation reactions including biomimetic bromination reaction.

Major objectives of the present research programme are as follows :

(i) To develop synthetic routes to newer stable peroxo complexes of vanadium stabilized by suitable co-ligands of biological relevance and to characterize them. It is of particular interest to obtain dinuclear compounds possessing bridging peroxo groups in addition to side-on bound peroxide.

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

(iii) To explore the catalytic activity of the compounds synthesized in bromide oxidation and bromination of organic substrates with an aim to pursue biomimetic chemistry of bromoperoxidase.

(iv) To undertake investigations involving studies on the interaction of the peroxo vanadium compounds as substrates for enzyme catalase, their possible inhibitory effect on certain enzymes and their redox activity with NADH.

Chapters 3 to 7 of the thesis present interpretative accounts of the results of our studies on the afore mentioned aspects of 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 8 some general conclusions drawn from the results of the work undertaken have been presented. Most of the new results have been published and the rest are under communication.

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CHAPTER 2

Materials and Methods

Materials and Methods

2.1 CHEMICALS

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

Vanadium pentoxide and vanadyl sulfate (SRL); hydrogen peroxide 30% (v/v), potassium bromide, potassium iodide, potassium hydrogen phosphates (E. Merck, India); ethylenediaminetetraacetic acid, nitrilotriacetic acid (SD fine chemicals); glucose oxidase (from *A. niger*), glycyl-peptides, phenol red, catalase, NADH (Sigma Alderich Chemicals Company Pvt. Ltd.); amino acids, sodium thiosulphate, potassium persulphate (CDH); HEPES Buffer (HiMedia); aniline, nitroanilines, aminophenols, quinol, 2-methoxytoluene, acetone, diethyl ether, ethyl acetate, petroleum ether 40°-60°C (SD fine chemicals).

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 Vanadium[1]

Vanadium was estimated volumetrically by titration with a standard potassium permanganate solution[1]. 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 *c.*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 *c.* 80°C, and finally titrated with a standard potassium permanganate solution.

2.2.2 Peroxide[2-4]

2.2.2.1 Permanganometry[2]

An accurately weighed amount of a peroxovanadate compound was dissolved in 7N sulphuric acid containing *c.*4g 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.

2.2.2.2 Iodometry[3]

To a freshly prepared 2N sulphuric acid solution, containing an appropriate amount of potassium iodide (~1g in 100 ml) was added an accurately weighed amount of peroxovanadate(V) compound with stirring. The mixture was allowed to stand for *c.*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.

2.2.2.3 By standard Ce(IV) solution[4]

An accurately weighed amount of 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. Vanadium(V) does not interfere in this method.

2.2.3 Sulphate[5]

A known amount of the sulphate compound of vanadium was dissolved in about 25 ml water and *c*.0.4 ml of conc. HCl was added to it. The solution was diluted to ~250 ml and boiled for *c*.30 min. To the boiling solution 10-12 ml of warm 5% barium chloride 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.

2.2.4 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 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 Laser-Raman (LR) spectra

The laser-Raman (LR) spectra were recorded on a SPEX Ramalog model 1403 Raman spectrometer. The 4880 A° laser line from a Spectra-Physics model 165 argon laser was used as the excitation source. The light scattered at 90^0 was detected with the help of a cooled RCA 31034 photomultiplier tube followed by a photon-count processing system. The spectra were recorded at ambient temperatures by making pressed pellets of the compounds.

2.3.7 Electron spin resonance (ESR) spectra

The electron spin resonance (ESR) spectra of aqueous solutions of the compounds were recorded at room temperature in a capillary tube in a Varian Model E 109 spectrometer under the following conditions : microwave power, 5 mW; microwave frequency, 9.05 GHz; modulation frequency, 100 KHz; modulation amplitude 4 ×1 G ; scan range 4 ×1 KG; field set 3200 G and receiver gain 2.5×10^3.

2.3.8 ^{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.

2.3.9 ^{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.

2.3.10 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).

2.3.11 Measurement of catalase dependent oxygen release[6]

A Gilson 5/6 H oxygraph fitted with a Clark oxygen electrode was used for measuring changes in dissolved oxygen in the medium (0.224 mM at 30°C) and the changes were recorded as units in μM of dissolved oxygen. The rate and the total amount of oxygen released from 0.2 mM solution of the compound in phosphate buffer (50 mM, pH 7.0) on adding catalase (0.08 mg protein/ml) were measured. The recorder pen was set in the middle of the chart paper for measuring the release of oxygen into the medium. The machine was standardized by the increases in dissolved oxygen obtained on adding catalase to buffered solutions containing known amounts of H_2O_2. Oxygen

transfer from H_2O_2 was complete within 10 sec. under theses conditions, but a maximum of only about 200 μM of O_2 can be measured before it comes out in the form of bubbles. This indeed limits the concentration of a substrate to 0.2 mM in this method.

2.3.12 Measurement of oxidation of NADH[7]

Weighed samples of the compounds (1-3mM) were added to phosphate buffer (50 mM, pH 7.0). At high concentrations of peroxovanadates used, their absorbance at 340-380 nm in the electronic spectra had to be balanced by adding equivalent amounts in the blank and experimental cuvettes. Only the experimental samples contained NADH (0.2 mM). Immediately after mixing, the sample was transferred to the cuvette and A_{340} was noted, and its decrease, indicating NADH oxidation, was followed with time.

2.3.13 Measurement of bromination activity in solution

The method of de Boer et al.[8] of introducing four bromine atoms into the molecule of phenol red (ε^{433} =19.7 mm) to form the product, bromophenol blue (ε^{592}=67.4 mm) 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 (2M) and phenol red (20 μM) kept at 30°C. The reaction was started by adding solid compounds and was monitored by the increase in absorbance at 592 nm of the product formed. The volume of the reaction mixture was kept at 25 ml in these experiments to

enable accurate weighing of small amounts of solid samples of peroxovanadate added. After mixing aliquots were immediately transferred to the spectrophotometer to record the increase in A_{592}.

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CHAPTER 3

Reaction of Diperoxovanadate with Vanadyl Sulphate in Presence of EDTA as an Access to Dinuclear Peroxovanadates(V)

activity[20,21]. An intermediate derived from diperoxovanadate was shown to stimulate oxidation of NADH[22]. It has been reported earlier by others[22-26] that diperoxovanadate (DPV) gains oxidant activity by complexing with vanadyl (V^{IV}), to form a highly reactive peroxo-bridged intermediate, $[OVOOVO(O_2)]^+$, which could act as oxidant species of NADH[22] and bromide[23], as well as inactivate glucose oxidase[24], or release oxygen as gas[25] in absence of any substrate.

One significant finding of such studies was the inhibitory effect of certain organic ligating agents viz., EDTA, histidine, imidazole etc. on the above redox processes[22-26]. Such inhibition was found to be maximum in presence of EDTA. EDTA induced inhibition[27] was also reported to occur in oxygen release reaction involving H_2O_2 and $VOSO_4$. The basis for such potent inhibition appeared to be the inactivation of the reactive peroxo intermediates by the ligands through complexation with V(V) and V(IV) centers. However, there has been a paucity of evidence on the exact identity of the actual species responsible for such inhibitory effect[22-26]. These findings were of interest to us as we were working at establishing viable synthetic routes to stable dinuclear peroxovanadates of biochemical relevance.

We therefore considered it worthwhile to investigate the reaction of DPV and VO^{2+} in presence of EDTA with an aim to ascertain the nature of the above inhibitory complex by attempting to isolate such species into solid state. We were particularly interested to explore the possibility of using the above reaction as a synthetic strategy to gain an access to novel dimeric heteroligand peroxo-vanadium compounds stable at physiological conditions. Since the proposed dimeric intermediate was a mixed valence vanadium species it also appeared possible to isolate a mixed-valence compound of vanadium by stabilizing it through complexation.

Moreover, it has been realised that any information related to interaction of vanadate or vanadyl with ligands like EDTA may be relevant in addressing the role of vanadium in vanadate mediated inhibition or activation of enzymes[3,4]. This ligand and related compounds are often additives in biological studies with vanadate[28].

In Chapter 3 of the thesis, an account of the reaction of alkali diperoxovanadate (ADPV) with $VOSO_4$ in presence of EDTA which led to the synthesis of novel dimeric peroxovanadates of the type $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$, A = Na or K (**3.1** and **3.2**) is presented. The compounds have been subjected to dissolution and reactivity studies in order to determine their nature, stability and redox properties in solution. This complex species is believed to correspond to the inhibitor complex involved in the above redox processes.

3.2 EXPERIMENTAL SECTION

The chemicals used were all reagent grade products (CDH, E Merck(India), SRL, SD Fine). Catalase and NADH were obtained from Sigma-Aldrich Chemicals Company Pvt. Ltd. The water used for solution preparations were deionised and distilled.

3.2.1 Preparation of alkali metal diperoxovanadate, $A[VO(O_2)_2(H_2O)]$ (A = Na or K)

Alkali metal diperoxovanadate (ADPV) was prepared by adding equal volume of H_2O_2 solution (40 mM) to a vanadate solution (20 mM) maintaining the pH at 7.0 by

adding dilute alkali hydroxide solution. On addition of pre-cooled ethanol to this solution, yellow microcrystalline product precipitated out which was separated by centrifugation, washed with ethanol and finally dried over conc. H_2SO_4. Analysis of the content of vanadium and peroxide agreed with the formula $A[VO(O_2)_2(H_2O)]$ (A = Na or K) .

3.2.2 Reaction of $A[VO(O_2)_2(H_2O)]$ (A = Na or K) with $VOSO_4$ in presence of EDTA. Synthesis of $A_4[V_2O_3(O_2)$ (EDTA) (SO_4) $(H_2O)].2H_2O$ (**3.1** and **3.2**)

In a typical reaction disodium or dipotassium salt of EDTA (2.5 mM) was dissolved in *c.*5 ml of water by warming. To this $VOSO_4.5H_2O$ (0.42g, 1.66 mM) was added with constant stirring. The reaction mixture was stirred for *c.*5 min in an ice-bath. Alkali hydroxide pellets were added to this solution to raise the pH to 7. Solid $A[VO(O_2)_2(H_2O)]$ complex (2.5 mM) was then added in one portion to the reaction mixture with constant stirring. The pH of the solution was ultimately raised to 8.5 by the further addition of AOH. The initial blue colour of the solution changed to green on addition of yellow ADPV and ultimately a deep red coloured clear solution was obtained within *c.*4 min. On addition of pre-cooled acetone in portions (*c.*5ml) to the reaction solution under vigorous stirring, a red colored pasty mass separated. The supernatant liquid was decanted off, and the oily residue was treated repeatedly with acetone under scratching until it became microcrystalline solid. The product was separated by centrifugation, washed with ethanol and dried *in vacuo* over conc. sulfuric acid.

3.2.3 Elemental analysis

Quantitative estimations of vanadium, peroxide, sulphate, 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.

3.2.4 Physical and spectroscopic measurements

Magnetic susceptibilities, molar conductances, UV-Vis, IR and ESR spectral measurements were done as per methods described in Chapter 2. Structurally significant IR bands and their assignments are reported in Table 3.2. The ^{1}H-NMR spectra were recorded in deuterium oxide using a Varian EM-390 90 MHz spectrophotometer. Sodium salt of 3-(trimethylsilyl)-1-propane sulphonic acid was used as the internal standard.

3.2.5 Stability of complexes in solution - measurement of oxygen release from the peroxo-vanadium complexes

A Gilson 5/6 H oxygraph fitted with a Clark oxygen electrode was used for measuring changes in the concentration of dissolved oxygen (0.224 mM at 30°C) in the medium by the method given in Chapter 2.

The effect of catalase on complexes was also studied by estimating the peroxide content of the compound **3.1** at different time intervals in a solution containing catalase. The reaction solution contained phosphate buffer (50 mM, pH 7.0), catalase (20 mg) and the compound **3.1** (50 mg). The volume of the reaction solution was kept at 25 ml.

Table 3.1. *Analytical data of the peroxovanadate compounds 3.1 and 3.2*

No.	Compound	(%calculated) % found							Approximate yield(%)
		Na/ K	C	N	H	V	O_2^{2-}	SO_4^{2-}	
3.1	$Na_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$	(12.92)	(16.85)	(3.93)	(2.52)	(14.32)	(4.49)	(13.48)	
		13.11	16.78	3.87	2.58	14.27	4.44	13.41	55
3.2	$K_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$	(20.10)	(15.46)	(3.60)	(2.31)	(13.14)	(4.12)	(12.37)	
		20.13	15.41	3.62	2.35	13.16	3.98	12.30	30

The solution was incubated at 30°C. Aliquots of 5 ml were pipetted out and titrated for peroxide content at time 5, 10, 20, 30 and 40 minutes of starting the reaction.

3.2.6 Measurement of activity in bromination and NADH oxidation

The method of de Boer et al.[29], of introducing four bromine atoms into the molecule of phenol red (ε^{433}mM = 19.7) to form the product, bromophenol blue (ε^{592}mM = 67.4), as described in Chapter 2, was used to measure bromination activity.

Activity of compounds in NADH oxidation was examined by using procedure mentioned in Chapter 2.

3.3 RESULTS AND INTERPRETATION

3.3.1 Isolation of the reaction products and characterization

The interesting findings regarding inhibitory effect of EDTA and other organic ligands observed in the case of DPV-V(IV) mediated redox processes[22-26], made us to realize the distinct possibility of stabilising and isolating the proposed dimeric peroxovanadate intermediate involved in these reactions, through complexation. Accordingly, reactions of alkali diperoxovanadate, EDTA and $VOSO_4$ were carried out at changing molar concentration ratios and varying pH, ranging from 7 to 9. The success of obtaining the solid dinuclear peroxovanadates containing SO_4^{2-} and EDTA as heteroligands, as sodium or potassium salts depended on the following essential components : maintenance of molar ratio of DPV : VO^{2+}: EDTA at 1:0.75:1, order of addition of the reactants, pH of 8.5, maintenance of required reaction time as well as

temperature at $\leq 4^0$C. A solid product isolated at pH 7-8 was found to be EDTA peroxo-vanadate, which rendered inconsistent analysis.

The complexes are microcrystalline products and are hygroscopic in nature. In the solid state they remained stable for several weeks when stored in sealed containers at temperature < 30°C.

The elemental analysis data provided crucial information regarding composition of the compounds. A ratio of 2:1 was ascertained for V: peroxide, V: EDTA, as well as for V: SO_4^{2-}. This suggested a dimeric nature of the complex species. The elemental analysis results and the molar conductance values obtained from measurement at ambient temperatures (510-522 $\Omega^{-1}cm^2mol^{-1}$) were in complete agreement with the formulation of the complexes as $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$, (A = Na or K).

The electronic spectra of the complexes **3.1** (*Fig. 3.1*) and **3.2** displayed a broad band at 390-400 nm ($\varepsilon_{mM} \approx 600$) which has been assigned to the peroxo (LMCT) transition. According to previous studies[25], the absorbance at 780 nm of blue coloured aqueous solution of $VOSO_4$ decreases progressively on adding batches of DPV to the solution. The complete absence of the 780 nm band in the spectra of the newly synthesised complexes (*Fig. 3.1*) indicated the oxidative loss of V^{IV} during complex formation. Occurrence of vanadium in the complexes **3.1** and **3.2** in its +5 oxidation state was further evident from their being diamagnetic at room temperature and ESR silent. The 8-band spectrum (hyperfine splitting **a** = 115G) characteristic of V^{IV} of an aqueous solution of $VOSO_4$ was not observed in case of the complexes (*Fig. 3.2*) suggesting oxidation of V^{IV} to V^{V} during the course of the reaction.

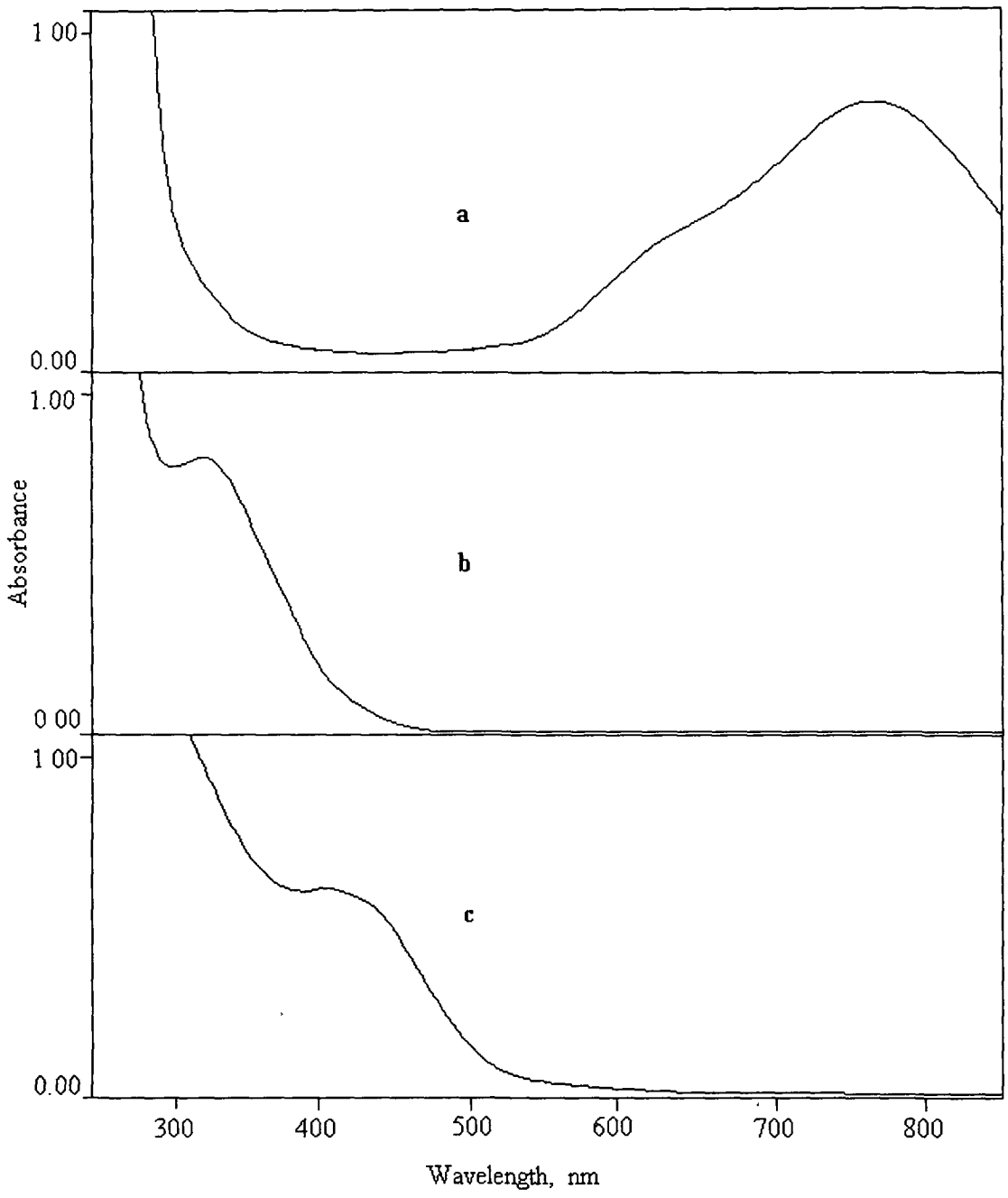

Fig. 3.1 UV-Vis spectra of aqueous solutions of reacting vanadium compounds and product. (**a**) Vanadyl sulphate (30 mM); (**b**) Na-DPV (1 mM); (**c**) compound **3.1** (0.5 mM). The characteristic absorbance of $VOSO_4.5H_2O$ at 780 nm is completely absent in the spectrum of the complex **3.1**.

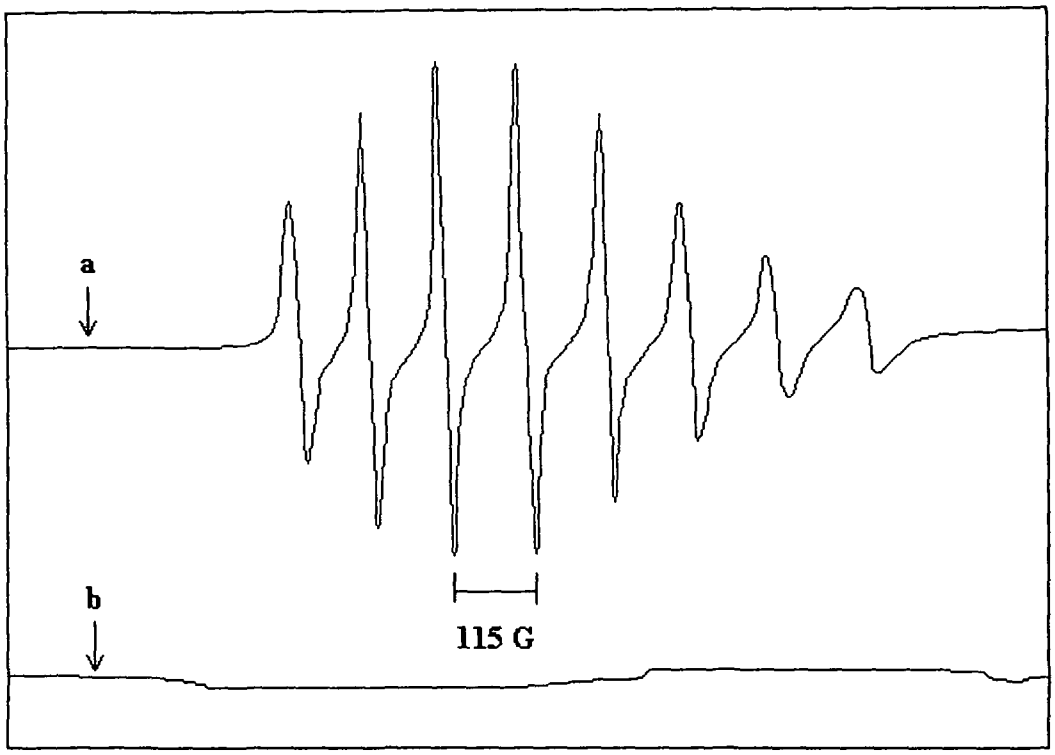

Fig. 3.2 ESR spectra of aqueous solutions of vanadyl sulphate and the dinuclear peroxovanadate. **a** - aqueous solution of $VOSO_4 5H_2O$, **b** - aqueous solution of peroxovanadate compound **3.1**.

The IR spectra of the complexes **3.1** (*Fig. 3.3*) and **3.2** displayed a rich but sufficiently well resolved spectral pattern significant features of which are summarized in Table 3.2. The ν(O-O) and the complementary ν(V-O_2) modes were observed in the positions stipulated for side-on bound peroxide[30-32]. For the binuclear V-O-V unit the antisymmetric and symmetric stretchings were expected in the 700 and 500 cm^{-1} region[32]. Accordingly, a medium intensity band observed at *c.*712 cm^{-1} was assigned to $\nu_{as}(V_2O)$ mode. The strong absorption at *c.*950 cm^{-1} was consistent with the presence of terminally bonded V=O group in the complexes. This band was observed to be rather

broad in the spectra probably owing to the presence of bridging oxo groups as well as co-ordinated sulphate in the complexes. The IR spectra (*Fig. 3.3*) of the complexes displayed a strong broad band with a maximum at *c.*1625 cm^{-1}, typical of co-ordinated carboxylato groups of EDTA[33]. The broadening of the band was possibly caused by the additional OH deformation modes of the water molecules present in the complexes. No other band was observed in the vicinity of 1700 cm^{-1} which indicated the absence of free carboxylate groups, thereby suggesting the co-ordination of EDTA as a hexadentate ligand in the complexes. The corresponding $\nu_s(COO^-)$ band of EDTA was observed at *c.*1399 cm^{-1} which was shifted from the free ligand value (1412 cm^{-1}) as expected for unidentate carboxylate groups[33]. The presence of water in the complexes was evident from the broad absorption at 3500-3400 cm^{-1}, due to ν(O-H). 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 *c.*755 cm^{-1} attributable to the rocking mode of water suggested the presence of co-ordinated water in each of the compounds.

The occurrence of co-ordinated sulphate in the complexes was evident from their IR spectra. When sulphate ion is co-ordinated to a metal, its IR spectrum changes drastically[34]. Free sulphate ion has T_d symmetry. It has four fundamental vibrations of which ν_3 and ν_4 are IR active. While the SO_4^{2-} ligand is bound in a mono-dentate fashion, its symmetry is lowered to C_{3v} due to which ν_3 and ν_4 are split into two bands each and both ν_1 and ν_2 appear with medium intensity. In case of bidentate co-

ordination of sulphate on the other hand, ν_3 and ν_4 are split into three bands each, while ν_1 and ν_2 still appear with medium intensity[34]. In the spectra of the compounds **3.1** and **3.2**, the splitting of the ν_3 and ν_4 modes of SO_4^{2-} into two bands each (Table 3.2) and presence medium intensity ν_2 mode at *c*.465 cm^{-1} were distinctly resolved. These observations cause us to infer that the sulphate ligand binds the vanadium centre in a unidentate (C_{3v}) fashion[34]. The ν_1 mode of a unidentately bonded SO_4^{2-} expected in the vicinity of 970 cm^{-1} could not be assigned decisively due to its possible mixing with the V=O stretching of terminal oxo groups.

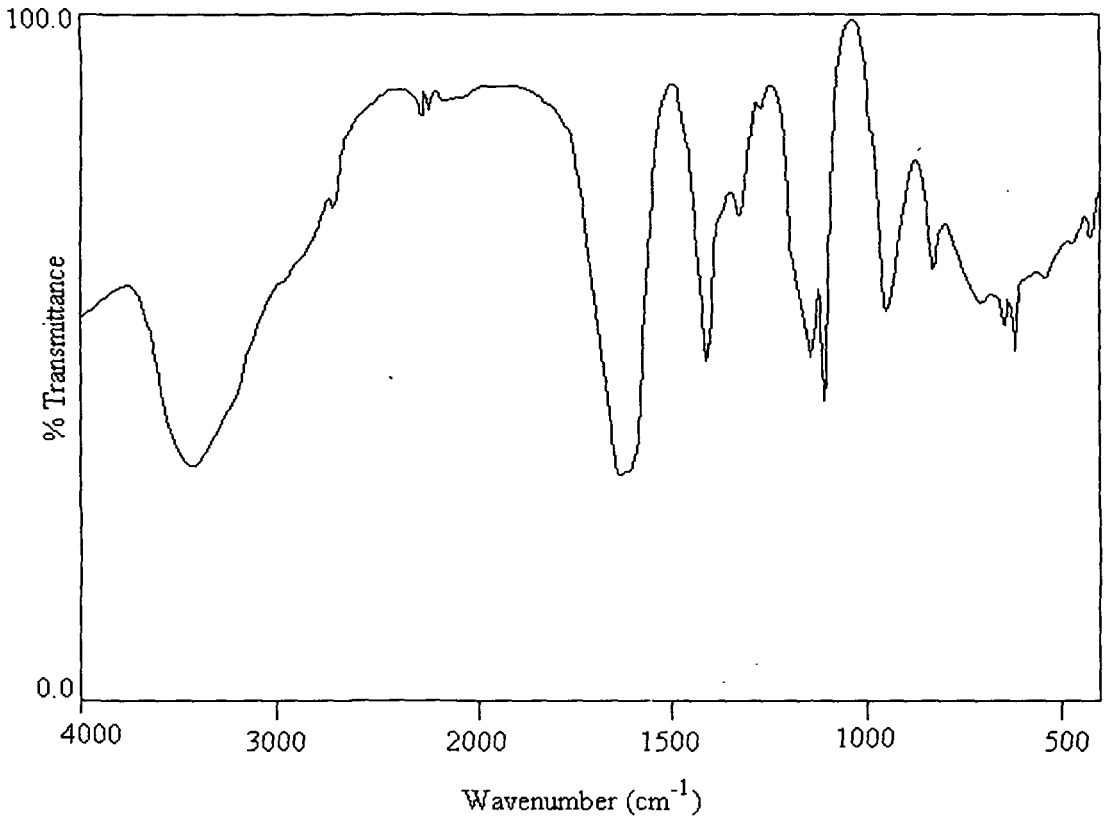

Fig. 3.3 IR spectrum of the complex **3.1**

Table 3.2. *Structurally significant IR bands of $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$ (A = Na or K)*

No.	Compound	IR bands cm^{-1}	Assignment
3.1	$Na_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$	3434m	ν(O-H)
		1624s	ν_{as}(COO)
		1404s	ν_s(COO)
		1136s 1103s	ν(S-O) (ν_3)
		945s	ν(V=O)
		836m	ν(O-O)
		755	$\rho_r(H_2O)$
		712m	$\nu_{as}(V_2O)$
		641m 614m	ν(S-O) (ν_4)
		584s	$\nu_s(V\text{-}O_2)$
		464m	ν(S-O) (ν_2)
3.2	$K_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$	3420m	ν(O-H)
		1631s	ν_{as}(COO)
		1399s	ν_s(COO)
		1138s 1105s	ν(S-O) (ν_3)
		944	ν(V=O)
		835	ν(O-O)
		755	$\rho_r(H_2O)$
		712	$\nu_{as}(V_2O)$
		638m 612m	ν(S-O) (ν_4)
		578	$\nu_s(V\text{-}O_2)$
		468m	ν(S-O) (ν_2)

The ^{1}H-NMR spectra of the complexes **3.1** and **3.2** exhibited a singlet at δ 2.71 and an AB quartret at δ 3.30 of intensity ratio 1: 2. On the basis of previous studies on EDTA complexes the AB quartret was assigned to the eight acetate protons and the singlet to the four ethylenic protons of the EDTA ligand[35]. The close analogy between the NMR spectra of the complexes **3.1** and **3.2** (*Fig. 3.4*) and that of a previously reported Mo-EDTA complex[35] containing bridging EDTA suggested the occurrence of the ligand in these complexes as a bridging one, as anticipated by us.

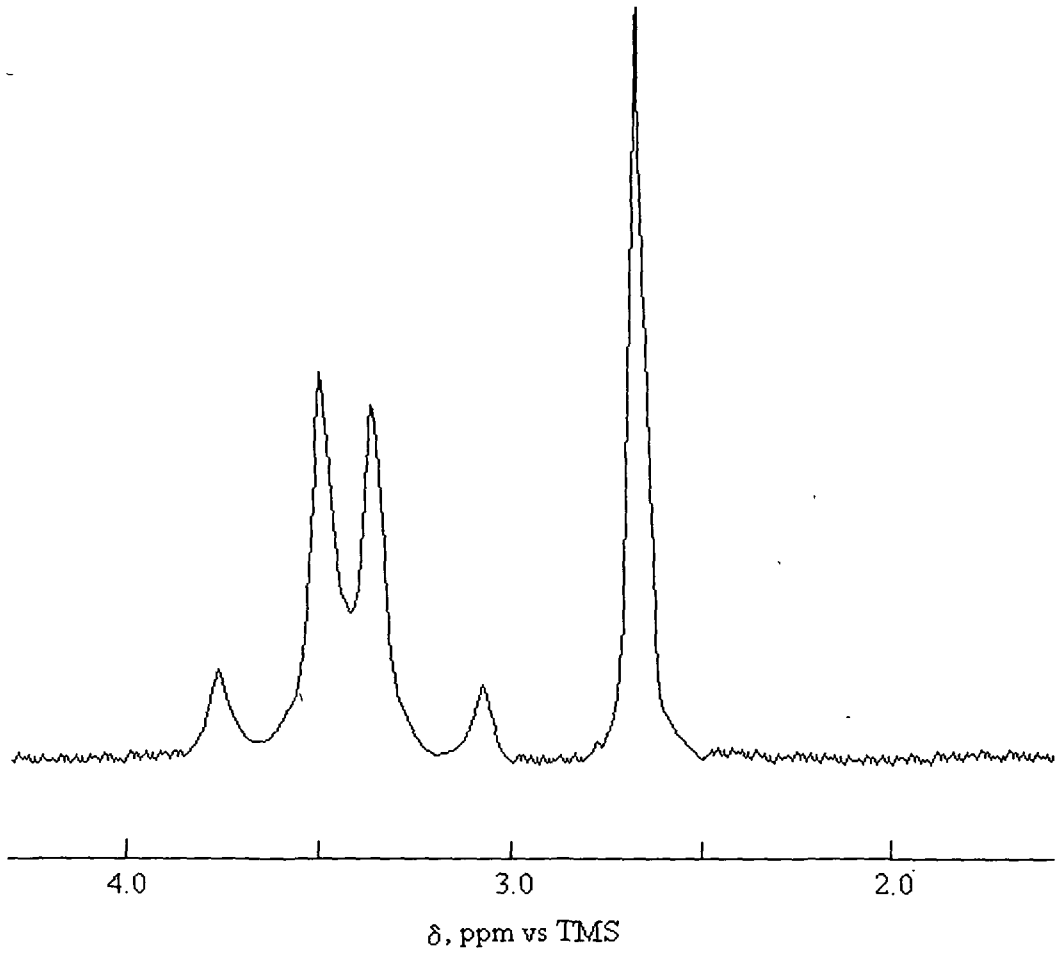

Fig. **3.4** ^{1}H-NMR spectrum of the complex **3.1**

The above results are consistent with a structure of the complex of the type shown in *Fig.3.5*. Hexadentate EDTA ligand occupying three co-ordination positions around each of the oxo-bridged hepta co-ordinated vanadium(V) centres probably enhances the stability of the dinuclear complexes.

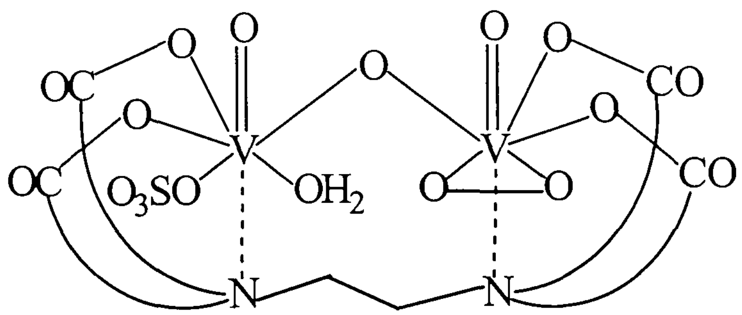

Fig. 3.5 Proposed structure of dinuclear heteroligand peroxovanadate(V) compounds, $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$ (A = Na or K)

3.3.2 Nature and stability of the complexes in solution

The stability of the complexes in solution has been studied by testing the possible oxygen release from a freshly prepared solution of the dinuclear peroxovandate **3.1** or **3.2** with the help of an oxygraph by adopting method described in

Chapter 2. However, no oxygen was found to be released on dissolution of the complex in water. Moreover, the single broad band observed in the 390-400 nm region in the electronic spectra of the complexes showed no change in its position or absorbance over a 2 hr period. From these observations in combination with molar conductance values the stability of the complexes in water was implicit.

3.3.3 Studies on redox properties of the compounds **3.1** and **3.2**

Having achieved the synthesis of these compounds we were interested in studying their activity in terms of their ability to oxidize bromide[23] and NADH[22], and their action with catalase[25], the enzyme that catalyze the breakdown of H_2O_2 formed during oxidative processes in the intercellular peroxisomes. On incubation with catalase DPV was found to be degraded releasing half the molecular equivalent of oxygen[36] at the rate of 36.0 μM/min from a solution of 0.2 mM. EDTA selectively inhibited this reaction of diperoxovandate with catalase[36]. For complex **3.1** and **3.2**, a maximum of 0.5 O_2 per mole of the compound is expected to be released due to the presence of one peroxide group per molecule. Surprisingly, no oxygen release took place from the solution of the complexes **3.1** and **3.2** in phosphate buffer (pH 7.0) on treatment with catalase and incubating at 30° C upto 30 min, confirming the resistance of the compounds to the enzyme. After 30 min, oxygen was found to be released from the solution at an extremely slow rate.

The complexes were unable to bring about NADH oxidation as expected. Under the conditions given above, NADH was rapidly oxidized[22] by a mixture of DPV and

$VOSO_4$. We now find that addition of the solid compound **3.1** to solution of NADH (0.2 mM) failed to oxidise it even at high concentration. There was no instant oxidation and A_{340} decreased at a negligible rate.

Unlike our experience with peroxo bridged dimeric compounds $[V_2O_2(O_2)_3L_2]$ (L = amino acid or peptide) which led to the instant bromination of phenol red into its 592-absorbing brominated product bromophenol blue[26] at physiological pH, as described in Chapter 5 of this thesis, with the complexes **3.1** and **3.2** no such activity was observed.

The above observations suggest that the factors such as absence of a μ-peroxo group in the complexes as well as their high stability due to chelation by EDTA are probably responsible for their lack of participation in the above redox processes thus resembling the inhibitor complex formed in solution.

3.4 DISCUSSION

Based on their detailed investigations on vanadyl-diperoxovanadate reactions[22-26] and taking into account the redox chemistry of vanadyl, vanadate and peroxo-vanadates described earlier by Brooks and Sicilio[27] and Jaswal and Tracey[17], it was proposed by Ramasarma et al. that a short lived $[OVOOVO(O_2)]^+$ species is the intermediate shared by the processes : oxidation of NADH[22], formation of oxidized bromine species[23], inactivation of glucose oxidase[24] and release of oxygen[25] (*Fig. 3.6*).

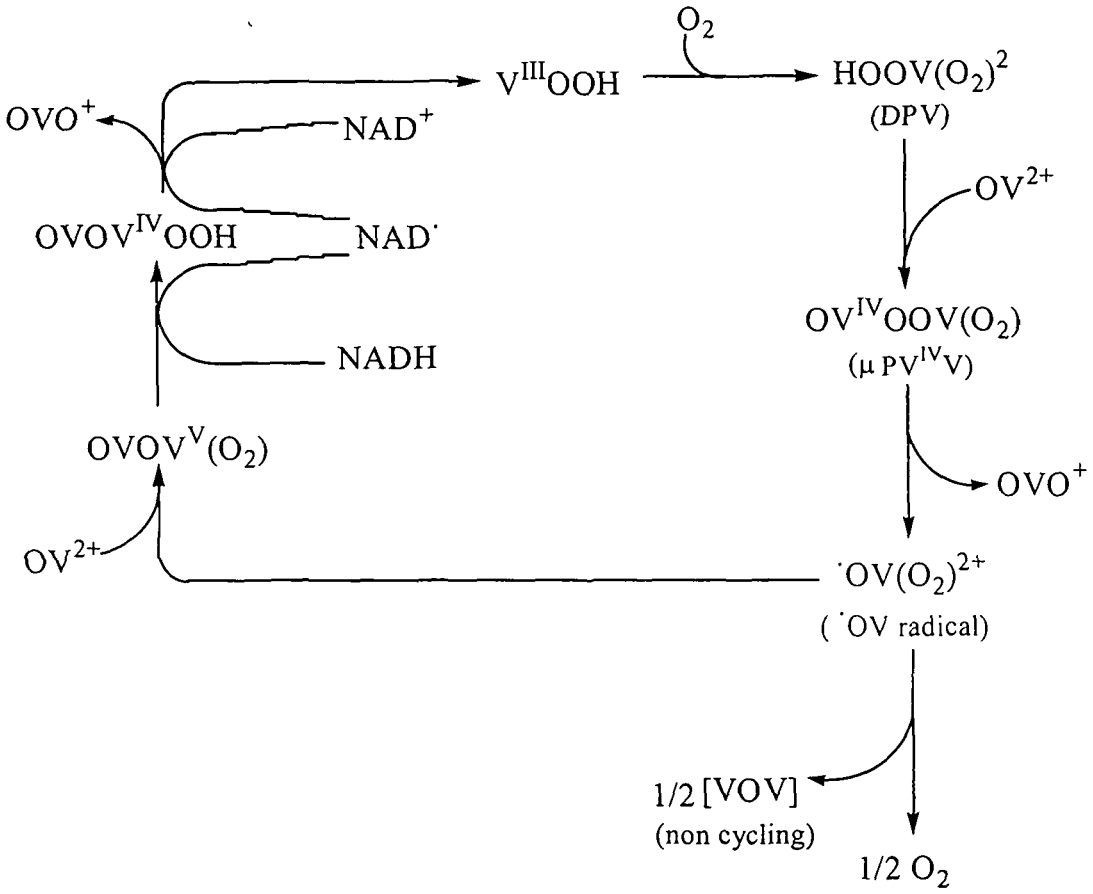

Fig. 3.6. Schematic representation of the profile of the possible reactions in diperoxovanadate-mediated interdependent oxidation of NADH and vanadyl and the accompanying oxygen exchanges[13].

The present investigation has established that it is possible to isolate the species formed in a solution of diperoxovanadate and VO^{2+} in presence of EDTA which is considered to be responsible for inhibiting the NADH and bromide oxidation and oxygen release reaction by a combination of DPV and VO^{2+}. To us it appears that

EDTA, which is known to undergo facile condensation reaction with DPV[3,4] and also forms stable complex with VO^{2+}, binds simultaneously to V(V) and V(IV) centres of the μ-peroxo-vanadate intermediate proposed to be formed in a solution of DPV and vanadyl (*Fig. 3.7*, complex I). This species then undergoes internal redox involving the reductive cleavage of the bridging peroxo group at the expense of oxidation of V(IV) to

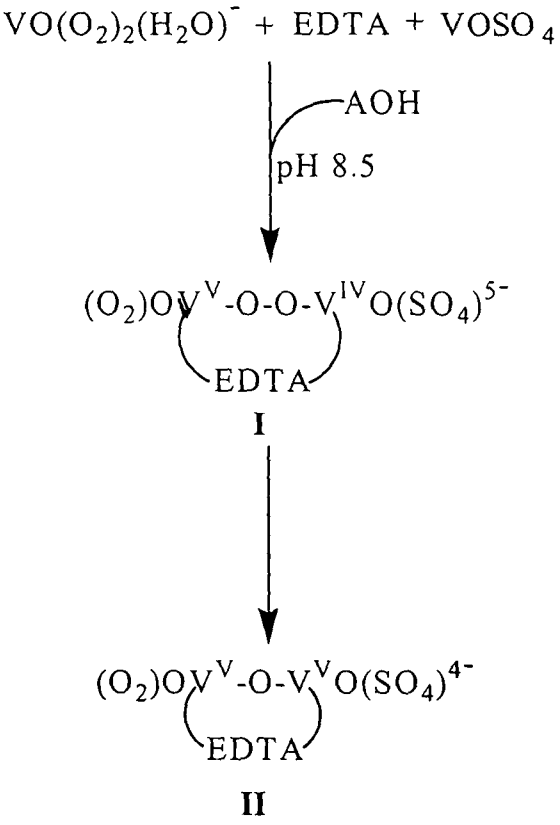

Fig. 3.7 Schematic representation of the formation of the dinuclear heteroligand peroxovanadate(V) complex, $[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)]^{4-}$ from the reaction of diperoxovanadate with $VOSO_4$ in presence of EDTA. Hexa co-ordination of EDTA is not shown for simplicity. No attempt is made to show exact stoichiometry of reactions.

V(V) leading to formation of the stable dinuclear EDTA and oxo bridged complex (*Fig. 3.7*, complex II), which is resistant to further degradative loss of peroxide and can be isolated in the solid state as its Na^+ or K^+ salts.

A significant finding in the present study is the high stability of the synthesized complexes at physiological pH and their resistance to catalase action. This may be relevant in the cellular milieu where H_2O_2 has little chance to survive abundant catalase and glutathione peroxidase. By forming peroxo complexes of the above type vanadate may provide a way of preserving cellular H_2O_2 in presence of abundant catalase and make it available for its functions.

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CHAPTER 4

Dinuclear Heteroligand Peroxovanadates(V): Synthesis, Characterization and Stability Towards Decomposition

Dinuclear Heteroligand Peroxovanadates(V): Synthesis, Characterization and Stability Towards Decomposition

4.1 INTRODUCTION

As a sequel to our investigation involving redox interaction of DPV with vanadyl in presence of EDTA, which afforded the isolation of novel dimeric peroxo compounds **3.1** and **3.2**, we considered it imperative to explore the synthetic utility of such reactions in the context of synthesis of newer members of this unique type of compounds, using other organic molecules as potential ligand in lieu of EDTA. In addition, it was felt that such studies will help in gaining further insight into the effect of organic molecules on DPV-V(IV) catalyzed redox processes[1-4].

A planning of synthetic strategies and working out of appropriate experimental conditions are important pre-requisites for the synthesis and characterization of new stable peroxo vanadium derivatives which exert a real attraction because of their possible pharmacological[5-9] and catalytic applications[10-15]. The ligands chosen for the present study required to possess the ability to form stable complexes with both peroxo-vanadium(V) as well as vanadyl so that it could bind to the DPV-V(IV) derived dimeric intermediate formed in solution. We decided to employ ligands which utilize functional groups as donor sites that are biologically relevant.

Many ligands of physiological interest contain more than one oxygen functionality and form strong complexes with vanadyl and vanadate[16]. Given the affinity of simple peptides for vanadyl cation[16-18] as well as its ability to co-ordinate with peroxovanadium moiety[19-22] prompted us to select gly-gly as one of the suitable

ligand systems for this investigation. Vanadium binds specifically and non-specifically to various proteins including carboxypeptidase, nucleases and phosphatses[16, 17]. Information regarding interaction of oxovanadium(IV) and vanadate with peptides in general, are important in the context of understanding the biological role of vanadium.

The tripodal amine, nitrilotriacetic acid (NTA) was chosen for this study because it is a ligand related to EDTA, and also it is known to form stable compound with peroxovanadate[23]. The NTA containing peroxovanadium compound $K_2[VO(O_2)NTA].2H_2O$ has been characterized structurally and it has shown remarkable insulin mimetic properties[24]. It is also reported that peroxovanadates with strongly chelating ligands like NTA and EDTA displayed better effect in terms of toxicity compared to other polycarboxylato heteroligand peroxovanadates when tested as drug against Murine Leukemia[6].

Chapter 4 of the thesis presents the successful synthesis of two new stable peroxovanadate compounds, $Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$ (**4.1**) and $Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$ (**4.2**), from the reaction of $Na[VO(O_2)_2(H_2O)]$ and $VOSO_4$ in presence of the respective co-ligands, and their characterization. Results of investigations on their stability towards decomposition in solution and interaction with catalase, NADH and bromide are also reported in this Chapter.

4.2 EXPERIMENTAL SECTION

The chemicals used were all reagent grade products (CDH, E Merck(India), SRL, SD Fine). Catalase, NADH and glycyl-glycine were obtained from Sigma-Aldrich

Chemicals Company Pvt. Ltd. and NTA was obtained from CDH. The water used for solution preparations were deionised and distilled. Na-DPV was prepared by method described in Chapter 3.

4.2.1 Reaction of $Na[VO(O_2)_2(H_2O)]$ with $VOSO_4$ in presence of nitrilotriacetic acid (NTA). Formation of dimeric peroxovanadate complex $Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$ (**4.1**)

A solution of NTA (0.28 g, 1.5 mM) was prepared by dissolving it in *c*.5 ml of water and raising the pH to *c*. 5 by dropwise addition of NaOH solution (conc. *c*.8 M). The solution was kept in an ice-bath. To this solution solid $Na[VO(O_2)_2(H_2O)]$ complex (0.172 g, 1 mM) was added in one portion with stirring. $VOSO_4.5H_2O$ (0.189 g, 0.75 mM) was added to this mixture with constant stirring maintaining the molar ratio of DPV : VO^{2+}: NTA as 1:0.75:1.5. The pH of the reaction solution, recorded at this stage was *c*.3 which was raised to *c*.7 by addition of concentrated NaOH solution which afforded a dark red coloured clear solution. To this solution when pre-cooled acetone (*c*.5ml) was added in portions a red colored pasty mass separated out under vigorous string. The supernatant liquid was decanted off, and the oily residue was treated repeatedly with acetone under scratching until it became microcrystalline solid. The product was separated by centrifugation, washed with ethanol and dried *in vacuo* over conc. H_2SO_4.

4.2.2 Reaction of $Na[VO(O_2)_2(H_2O)]$ with $VOSO_4$ in presence of glycyl-glycine. Formation of dimeric peroxovanadate complex $Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$ (4.2)

The ligand glycyl-glycine (0.2g, 1.5 mM) was dissolved in *c.*5 ml of water. To this solution $VOSO_4.5H_2O$ was added with constant stirring maintaining the molar ratio of gly-gly: VO^{2+} as 1.5: 0.5. The reaction mixture was then stirred for *c.*5 min in an ice-bath. During this period the solid $VOSO_4.5H_2O$ dissolved yielding a blue colored solution. At this stage solid $Na[VO(O_2)_2(H_2O)]$ complex (0.172g, 1mM) was added to the reaction mixture in one portion with constant stirring. The pH of the solution was ultimately raised to *c.*9 by adding concentrated sodium hydroxide solution (conc. *c.*8M). On addition of yellow NaDPV, the initial blue color of the solution changed to green and then to deep red coloured clear solution within *c.*5 min. To the resultant red coloured solution when pre-cooled acetone in portions (*c.*5ml) was added with vigorous string, a red coloured pasty mass separated out. The supernatant liquid was decanted off, and the oily residue was treated repeatedly with acetone under scratching until it became microcrystalline solid. The product was separated by centrifugation, washed with ethanol and dried *in vacuo* over conc. H_2SO_4.

4.2.3 Elemental analysis

Quantitative estimations of vanadium, peroxide, sulphate, carbon, hydrogen, nitrogen, and sodium were accomplished by methods described in Chapter 2. The analytical data of the compounds are summarized in Table 4.1.

Table 4.1. *Analytical data of the peroxovanadate compounds **4.1** and **4.2***

No.	Compound	(% calculated) % found							Approximate yield(%)
		Na	C	N	H	V	O_2^{2-}	SO_4^{2-}	
4.1	$Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$	(16.19)	(16.90)	(3.28)	(1.40)	(11.97)	(3.75)	(11.26)	
		16.25	17.01	3.37	1.51	12.05	3.61	11.13	50
4.2	$Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$	(7.18)	(15.00)	(8.75)	(2.18)	(15.93)	(5.00)	(15.00)	
		7.27	15.07	8.63	2.29	15.99	4.90	14.91	45

4.2.4 Physical and spectroscopic measurements

Physical and Spectroscopic measurements were performed by using instruments and procedures described in Chapter 2. Structurally significant IR and UV bands and their assignments are reported in Table 4.2.

4.2.5 Studies on stability in solution and redox activity of compounds

Measurement of oxygen release from the compound (± catalase) and their possible activities in oxidation of bromine and NADH were carried out by procedures outlined in Chapters 2 and 3.

4.3 RESULTS AND INTERPRETATION

4.3.1 Isolation of the reaction product and characterization

The methodology for the syntheses of the title compounds, which was not very different from the one adopted for the synthesis of compounds **3.1** and **3.2**, was based on the reaction of alkali diperoxovanadate and $VOSO_4$ in presence of the respective ligands, in water, at pH *c.*7 (for **4.1**) and *c.*9 (for **4.2**). As in the case of the synthesis of compounds **3.1** and **3.2**, sequence of addition of the reactants as well as maintenance of the molar ratio of DPV: VO^{2+}: NTA as 1:0.75:1.5 and that of DPV: VO^{2+}: gly-gly at 1: 0.5: 1.5 was found to be equally important for achieving the desired syntheses. Our attempts to isolate such complex species in presence amino acids like histidine, alanine and proline were unsuccessful.

The pH of the solution was critical for the outcome of the reaction run. Slight variation of the pH or other reaction conditions led to the formation of vanadate products devoid of peroxide with inconsistent analysis. The employed alkali hydroxide also provided the necessary cations to counterbalance the charge of the anionic complexes generated and subsequently isolated.

The results of elemental analyses on the isolated micro-crystalline reaction products and their molar conductance value of 735 Ω^{-1} cm^2mol^{-1} (for **4.1**) and 263 $\Omega^{-1}cm^2mol^{-1}$ (for **4.2**) (Table 4.2) are consistent with their formulations as $Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$ and $Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$.

The progress of the afore-mentioned redox reactions could be conveniently monitored in solution by studying the characteristic colour changes taking place during the course of the reactions with the help of UV-Vis spectroscopy. The absorbance at 780 nm of the blue coloured solution containing $VOSO_4$ and the ligand rapidly decreased on addition of the yellow solution of NaDPV and was lost completely within *c.*5 min of starting the reaction with a concomitant appearance of a weak intensity band in the 390-420 nm region (*Fig. 4.1*). The oxidative loss of V(IV) during the complex formation was thus apparent from these observations. The electronic spectra of the compounds **4.1** and **4.2** in aqueous solution displayed a broad band at 422 nm ($\varepsilon_{mM} \approx 766$) and 395 nm ($\varepsilon_{mM} \approx 432$), respectively which was assigned to peroxo to vanadium (LMCT) transition (Table 4.2). The band was observed in the range characteristic of a monoperoxovanadate(V) species[23]. The compounds were observed to be diamagnetic and ESR silent suggesting the complete oxidation of vanadyl to V(V) during the reactions.

Table 4.2. *Molar conductance value and electronic spectral data of the peroxovanadate complexes **4.1** and **4.2***

No.	Compound	Peak		Molar conductance
		nm	A(1mM)	$\Omega^{-1}cm^2mol^{-1}$
4.1	$Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$	422	0.76	735
4.2	$Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$	395	0.43	263

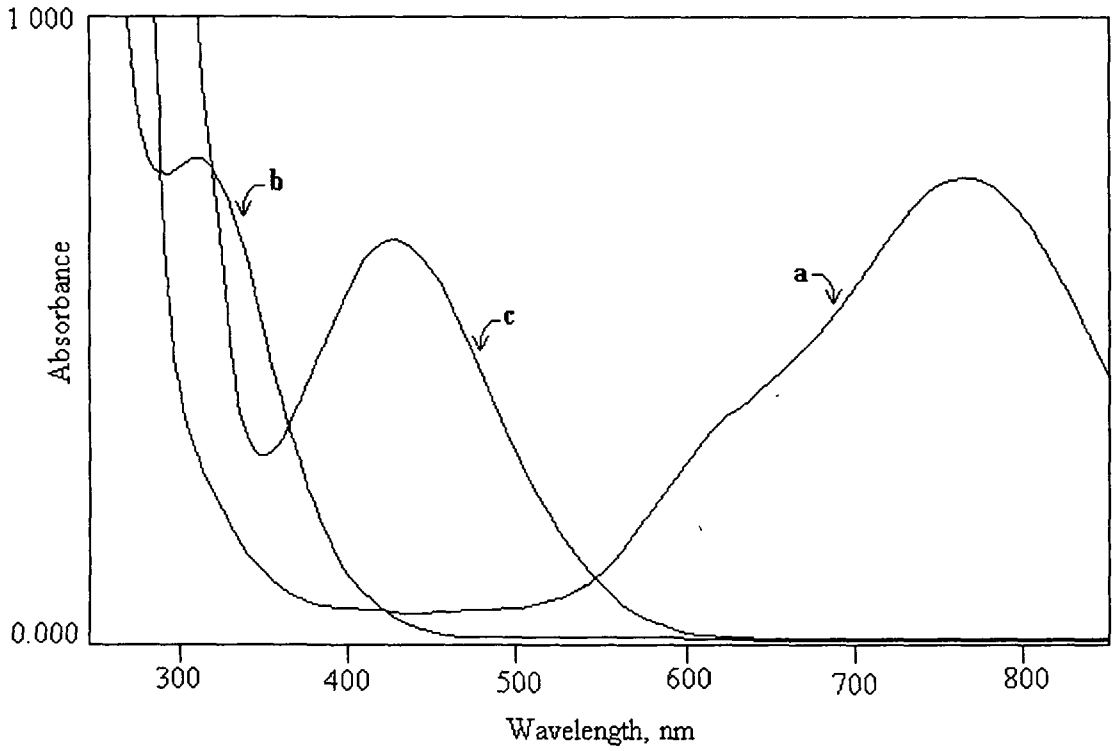


Fig. 4.1. UV-Vis spectra of aqueous solutions of vanadium compounds. (**a**) Vanadyl sulphate (30 mM); (**b**) Na-DPV (1 mM); (**c**) compound **4.1** (0.9 mM). The characteristic absorbance of $VOSO_4$ at 780 nm is completely absent in the spectrum of the reaction product, complex **4.1**.

Each of the title compounds exhibited characteristic spectral pattern in the infrared region (*Fig. 4.2* and *Fig. 4.3*). The spectra evidenced for the presence of co-ordinated oxo, peroxo and co-ordinated sulphate groups in each of the complexes. The bands for these groups displayed a close analogy with those of compounds $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$ (A = Na, K), in their position and pattern. The significant general features are presented in Tables 4.3 and 4.4. The strong absorption at *c.*950 cm^{-1} and a medium intensity one at *c.*710 cm^{-1} have been attributed to $\nu(V=O)$ and $\nu_{as}(V_2O)$ modes of terminal V=O and bridging V-O-V units, respectively[25]. The spectral pattern originating from peroxo group was characteristic of side-on bound peroxide[23,25,26] (Table 4.3 and 4.4). The well-resolved splitting of the ν_3 and ν_4 modes of SO_4^{2-} into two bands each (Table 4.3 and 4.4) at *c.*1180-1110 and *c.*640-610 cm^{-1}, respectively and appearance of medium intensity ν_1 and ν_2 mode at *c.*930 and *c.*430 cm^{-1} conclusively proved that the sulphate ligand binds the vanadium centre in a unidentate (C_{3v}) fashion[27]. The broad absorption displayed at *c.*3500-3400 cm^{-1} in the spectrum of each of the complexes was assigned to $\nu(OH)$ vibration of water molecule. A band at *c.*755 cm^{-1}, assigned to the rocking mode of water suggested the occurrence of co-ordinated water.

In addition to the features originating from VO^{3+}, V_2O, co-ordinated peroxide and sulphate groups, the complex **4.1** showed strong absorptions at 1644 and 1405 cm^{-1} due to the co-ordinated nitrilotriacitic acid (NTA) ligand. The spectral pattern compared very well with that reported for the co-ordinated NTA system where all the three carboxylate groups are bonded to the metal center[28,29].

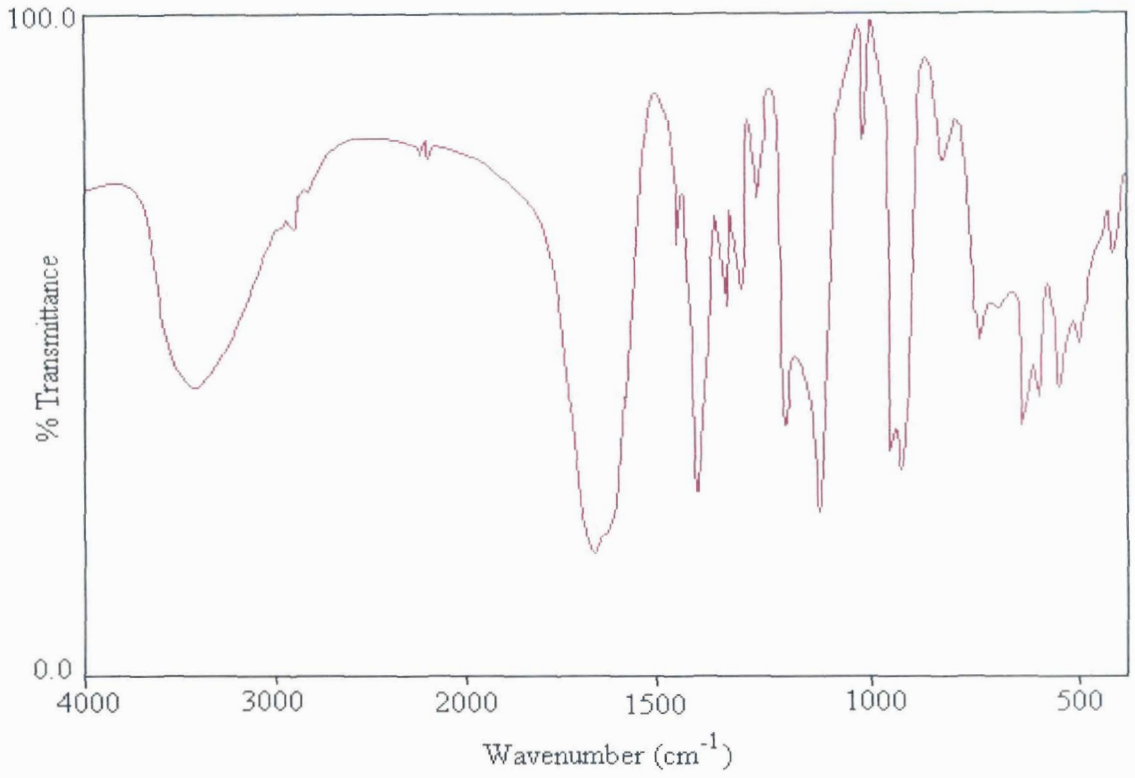


Fig. 4.2. IR spectrum of $Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$.

Table 4.3. *Structurally significant IR bands of* $Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$

No.	Compound	IR bands cm^{-1}	Assignment
4.1	$Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$	3500-3400	ν(O-H)
		1644s	ν_{as}(COO)
		1405s	ν_s(COO)
		1189s, 1121s	ν(S-O) (ν_3)
		945	ν(V=O)
		926	ν(S-O) (ν_1)
		833	ν(O-O)
		755	$\rho_r(H_2O)$
		707	$\nu_{as}(V_2O)$
		636m, 616m	ν(S-O) (ν_4)
		559	$\nu_s(V\text{-}O_2)$
		428m	ν(S-O) (ν_2)

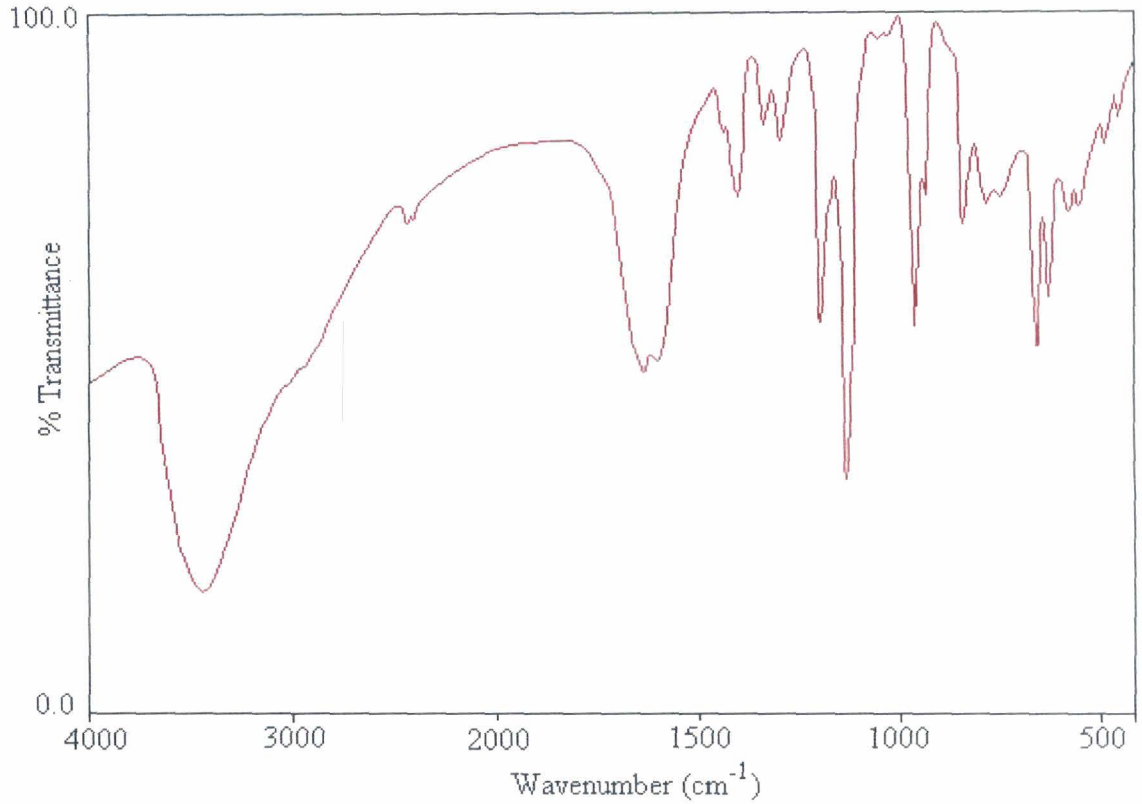


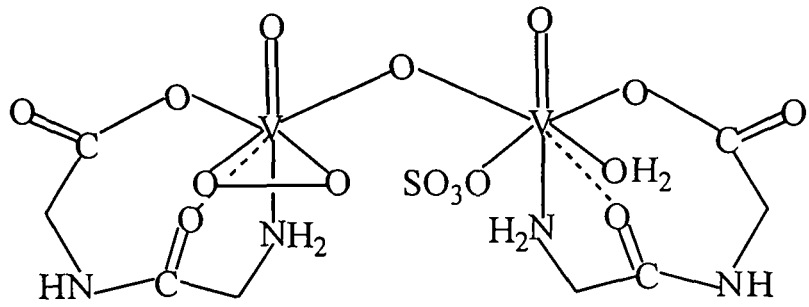
Fig. 4.3. IR spectrum of $Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$.

Table 4.4. *Structurally significant IR bands of* $Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$

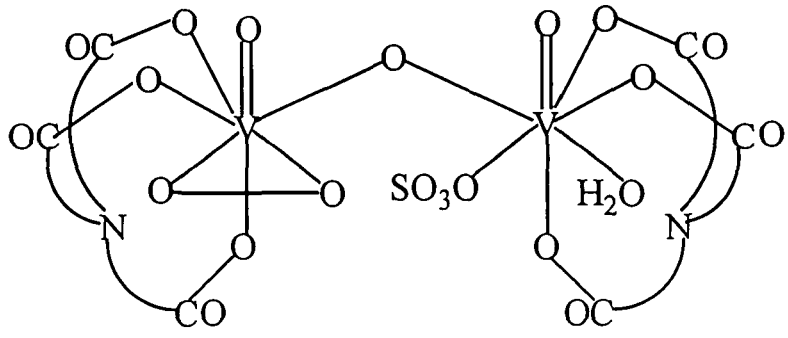
No.	Compound	IR bands cm^{-1}	Assignment
4.2	$Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$	3500-3400	ν(O-H)
		1360	ν_s(COO)
		1184s, 1119s	ν(S-O) (ν_3)
		955	ν(V=O)
		931s	ν(S-O) (ν_1)
		833m	ν(O-O)
		755	$\rho_r(H_2O)$
		712m	$\nu_{as}(V_2O)$
		631m, 620m	ν(S-O) (ν_4)
		578s	$\nu_s(V\text{-}O_2)$
		426m	ν(S-O) (ν_2)

In case of the dipeptide containing complex **4.2**, a strong but broad absorption was observed at *c.*1624 cm^{-1} and a medium intensity band at 1360 cm^{-1}. A simple peptide can act as a mono, bi- or tridentate ligand with different combinations of its binding sites[21,30-35]. The infrared spectra of gly-gly and its compounds have been extensively studied in solution[36-40] as well as in solid state[40]. In free gly-gly ligand ν(C=O) (amide), ν_{as}(COO) and ν_s(COO) modes of vibrations are observed at 1673, 1598 and 1405 cm^{-1}, respectively. On the basis of the available IR data[40-42] on co-ordinated gly-gly, the absence of bands in the 1670 cm^{-1} and 1570 cm^{-1} region characteristic of free amide carbonyl and $\delta(NH_2)$ groups and observance of the 1624 cm^{-1} band may be safely interpreted as an indication of the ligand, in its anionic form, being co-ordinated through O(amide) and -NH_2 groups. Participation of N(amide) in co-ordination appears to be unlikely since it is known to cause much larger decrease in peptide carbonyl stretching frequency[34,40] than observed in the present case. Involvement of carboxylate group in co-ordination was evident from the shifting of the ν_s(COO) to a lower frequency of 1360 cm^{-1} compared to its free ligand value, although the corresponding antisymmetric stretching could not be assigned decisively due to its combining with the band at 1624 cm^{-1} region.

The structures envisaged for these complexes on the basis of the above data are presented in *Fig. 4.4(a)* and *4.4(b)*. The ligands, NTA in complex **4.1** and gly-gly in compound **4.2**, occurring in their anionic tridentate forms apparently complete hepta co-ordination around each vanadium(V) center lending stability to the dinuclear complex species.



(a)



(b)

Fig. 4.4 Proposed structures of dinuclear heteroligand peroxovanadate(V) compounds. **(a)** $Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$, **(b)** $Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)\ (H_2O)].2H_2O$.

4.3.2 Investigation on stability and reactivity of the complexes **4.1** and **4.2** in solution

One of the primary interests of this study was to isolate peroxovanadate 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[43]. In order to determine their stability in solution, the title compounds were

tested for possible oxygen release with the help of an oxygraph. Furthermore, their peroxide contents and molar conductances as well as absorbances at 390-420 nm region in the electronic spectra were examined at different time intervals for any possible change. Like in the case of dinuclear complex species, **3.1** and **3.2**, the above investigations revealed that oxygen was not released on dissolution of the complexes in water and their peroxide content, electronic spectral band and molar conductance values remained unaltered over a period of 2 hrs. These results attest to the stability of the complex species in solution.

No oxygen was found to be released from the compounds and their peroxide content remained unchanged on treatment with catalase. The resistance of the compounds to the enzyme was thus evident. The complexes were inactive in oxidation of NADH and bromide thereby lending further credence to the proposal that this type of species would probably be involved in the inhibition of DPV-V(IV) mediated redox processes[1-4] in presence of certain organic molecules with the ability to form chelates.

4.4 DISCUSSION

Synthesis of two new members of a unique type of heteroligand peroxovanadate compounds, $Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$ and $Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$ have been achieved by developing an unusual, yet viable synthetic methodology. Peroxovanadate complexes are generally synthesized from the condensation reaction of vanadate and hydrogen peroxide and the number of peroxo groups per vanadium center usually increases with the increase in pH of the reaction solution[26,44,45]. No Information appears to be available regarding the

synthesis of monoperoxovanadate complexes from solution of alkaline pH. It is plausible that presence of tridentate NTA and gly-gly ligands in compounds **4.1** and **4.2**, respectively and a hexadentate EDTA occupying three co-ordination position around each vanadium in compounds **3.1** and **3.2**, are responsible for the presence of monoperoxo vanadate moiety in these compounds. This is also in agreement with earlier reports where it was observed that formation of monoperoxo-vanadate species are usually favored in presence of a tridentate co-ligand[21,45]. Thus denticity of the co-ligand also seems to play an important role in the isolation of the newly synthesized dinuclear compounds.

Significantly, the IR spectral pattern originating from co-ordinated sulphate in the complexes was observed to be very similar to that of vanadyl sulphate, $VO(SO_4).5H_2O$. This observation made us to infer that the sulphate-vanadium co-ordination in $VOSO_4$ remained unaltered during its reaction with DPV in presence of co-ligands (EDTA, gly-gly or NTA) neither being affected by the oxidation of V^{IV} to V^{V} nor by complexation with the respective co-ligand, leading to the formation of the newly synthesized complexes. Involvement of a dimeric intermediate in the above redox processes is evident from the results and the dimeric product obtained. The observations are supportive of the reaction pathway proposed for the formation of complex **3.1** and **3.2** (*Fig. 3.7*).

Thus it may be concluded that the reaction of DPV with $VOSO_4$ may serve as a paradigm for the synthesis of stable dinuclear peroxovanadates if carried out in presence of suitable ligands of appropriate denticity. It is also evident *inter alia* that DPV species in combination with $VOSO_4$ offers potential as novel synthon.

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CHAPTER 5

Synthesis and Characterization of Peroxo-bridged Divanadates with Peptides as Heteroligands. Studies on Their Nature and Stability in Solution

Synthesis and Characterization of Peroxo-bridged Divanadates with Peptides as Heteroligands. Studies on Their Nature and Stability in Solution*

5.1 INTRODUCTION

The importance of and the interest in peroxovanadium 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, as well as in Chapters 3 and 4. Our interest in the design, synthesis and study of peroxovanadate compounds has been spurred by the increasing importance of heteroligand peroxovanadates mainly attributable to their potential as models for understanding vanadium dependent biogenic systems[1,4-7].

A large number of peroxovanadium and oxodiperoxovanadium(V) complexes in diverse ligand environment have been structurally characterized in recent years[1,2,8-11] Majority of synthetic heteroligand peroxo-complexes of V(V) represent anionic mono, di or tetra peroxo complexes containing peroxo group bonded to V in a side-on fashion[1,2,9]. 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[1,2,9].

Dinuclear peroxovanadate compounds with various bridge configurations are known in which either an oxo group or a donor atom of the heteroligand usually binds the two vanadium centers[8,9]. Peroxo vanadium species containing bridging peroxo

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* Results described in this Chapter have been published in :
(i) *J. Chem. Res (M)*, 2001, 0536.
(ii) *Mol. Cell. Biochem*, 2002, **236**, 95.

group have been known to exist in solution and implicated as intermediate in some biochemical processes[3] as mentioned in Chapters 3 and 4. The existence of a dimeric species of the type, $(VO)_2(O_2)_3$ in an acidic solution and its being the possible critical oxidant of bromide has been reported[6] although, its exact identity is yet to be ascertained in solution[12]. A perusal of available literature on peroxovanadium chemistry however, indicates that reports related to synthesis and reactivity of dinuclear peroxo vanadium complexes containing bridging peroxogroup are very limited[13-16] and is an area needing exploration.

Moreover, despite the large number of heteroligand complexes that has been synthesized in recent years[1,2,8-11] and the intense biological work and solution studies carried out on interaction of vanadates with biogenic species viz., amino acids, peptides and proteins[17-21], information pertaining to well characterized synthetic peroxo-vanadium complexes with co-ordinated peptides are very few[22-24]. Peptides are probably the primary ligands to interact with vanadyl and vanadate in biological systems. A better understanding of the complexation behaviour of vanadium with such ligands is therefore of vital interest. Thus the paucity of information on peroxo-bridged divanadates as well as peptide peroxovanadates and the current intensive search for bio-relevant vanadium complexes[4,5,25,26], prompted us to direct our efforts in establishing viable synthetic routes to newer dimeric peroxovanadates stabilized by biogenic ligands viz., di- and tripeptides.

The present Chapter reports the synthesis and physicochemical characterization of a series of novel peroxovanadate complexes with distinctive features of having a μ-peroxo group and dipeptides or a tripeptide as heteroligand of the type, $[V_2O_2(O_2)_3(\text{dipeptide})_3].H_2O$ [dipeptide = gly-gly (**5.1**), gly-ala (**5.2**), or gly-asn (**5.3**)]

and $[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2].H_2O$ (**5.4**). Also reported in this Chapter are the results of studies on the nature and stability of the complexes in solution and their interaction with the enzyme catalase.

5.2 EXPERIMENTAL SECTION

The chemicals used were all reagent grade products (SD fine chemicals, E. Merck (India), SRL, CDH). Catalase and glycyl-peptides were obtained from Sigma-Aldrich Chemicals Company Pvt. Ltd. The water used for solution preparation was deionised and distilled.

5.2.1 Synthesis of peroxo-bridged vanadium(V) complexes with peptides as heteroligands

The common procedure for the synthesis of peroxovanadate complexes of peptides consisted of adding H_2O_2 (30% solution, 15 ml, 132.3 mM) gradually with continuous stirring to a mixture of solids of V_2O_5 (0.25g, 1.37 mM) and the peptides with a molar ratio of V: ligand of 2: 3. The mixtures were cooled in an ice-bath and kept stirred for about 15 min by which time the solids dissolved yielding red-coloured solutions. These solutions were all acidic and their pH was recorded to be 2.0 or below. No attempt was made to adjust pH in these experiments. On adding pre-cooled ethanol (about 15 ml) to these mixtures under continuous stirring, an orange-coloured pasty mass separated at this stage. After standing for about 15 min in the ice bath, the supernatant liquid was decanted, and the residue was treated repeatedly with

acetone : ethanol (3 : 1, v/v) mixture under scratching until it became micro-crystalline solid. The product was separated by centrifugation, washed with cold ethanol and dried *in vacuo* over concentrated H_2SO_4 The yields were in the range of 32-50% on weight basis.

5 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 are summarized in Table 5.1.

5.2.3 Spectroscopic measurements

Spectroscopic measurements were performed by using instruments and methods described in Chapter 2. Structurally significant IR and UV bands and their assignments are reported in Table 5.2.

5.2.4 Stability of complexes in solution - measurement of catalase dependent oxygen release from the peroxo-vanadium complexes

A Gilson 5/6 H oxygraph fitted with a Clark oxygen electrode was used for measuring changes in the concentration of dissolved oxygen (0.224 mM at 30^0C) in the medium by the method given in Chapter 2. Results of oxygen release reactions are presented in Table 5.3.

Table 5.1. *Analytical data of synthesized peroxovanadate complexes 5.1-5.4*

No.	Compound	(% calculated) % found					Approximate yield(%)
		C	N	H	V	O_2^{2-}	
5.1	$[V_2O_2(O_2)_3(gly\text{-}gly)_3].H_2O$	(22.26)	(12.98)	(4.17)	(15.77)	(14.84)	
		22.80	13.15	4.29	15.90	15.00	50
5.2	$[V_2O_2(O_2)_3(gly\text{-}ala)_3].H_2O$	(26.24)	(12.24)	(4.37)	(14.84)	(13.99)	
		22.35	12.30	4.40	14.60	14.80	41
5.3	$[V_2O_2(O_2)_3(gly\text{-}asn)_3].H_2O$	(26.50)	(15.46)	(4.05)	(12.52)	(11.78)	
		27.10	14.80	4.20	11.67	12.20	32
5.4	$[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2]H_2O$	(23.00)	(13.42)	(3.51)	(16.29)	(15.34)	
		23.40	13.90	4.00	16.60	15.25	52

Stability in solution was also ascertained by estimating the peroxide content in aliquots drawn from a standard solution of the compound at different time intervals. The stability of the compounds in solid state were determined by the estimation of their peroxide content periodically.

5.3 RESULTS AND INTERPRETATION

5.3.1 Synthesis and characterization

The essential components of the methodology on which success of synthesis of the title compounds **5.1-5.4** depended, include the use of an acidic medium, presence of water limited to that contributed by 30% H_2O_2, maintenance of reaction temperature at $\leq 4^0$C. The appropriate pH for the successful synthesis of these molecular compounds was ascertained to be *c*.2. Strategically, the reactions were carried out in absence of alkali mainly in order to avoid the presence of counter cations in solution. The glycyl-peptides with hydrophobic amino acids, valine and leucine failed to give a solid product under similar conditions. Our attempts to obtain suitable crystals of these compounds for structural studies have not been successful so far.

The orange coloured microcrystalline compounds **5.1-5.4** are all hygroscopic in nature at ambient conditions and decompose in a few days. However, the compounds were found to be stable for several weeks stored dry at <20°C. Magnetic susceptibility measurements revealed diamagnetic nature of the compounds in conformity with the presence of vanadium in its +5 oxidation state in each of them.

Valuable information regarding the composition of the compounds was obtained from the elemental analyses data (Table 5.1). The $V:O_2^{2-}$ ratio in each of the complexes was unequivocally ascertained to be 2:3. This suggested a dimeric nature of the compounds presumably, involving a bridging peroxide group. The C, H, N analysis results of complexes **5.1-5.3** revealed the V: dipeptide ratio to be 1: 1.5 or 2:3 whereas, this was found to be 1:1 for the tripeptide containing complex **5.4**. These results were compatible with the general molecular formula, $[V_2O_2(O_2)_3(dipeptide)_3].H_2O$ for compounds **5.1-5.3** and $[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2].H_2O$ for compound **5.4.**

The IR spectra of the complexes **5.1-5.4** gave clear indication of the presence of co-ordinated peroxide, co-ordinated peptide and terminally bonded V=O groups and lattice water in each of them (*Fig. 5.1, 5.3, 5.5* and *5.7* and Table 5.2). The strong absorption at *c.* 960–930 cm^{-1} in the spectra of each of the complexes was consistent with the presence of terminally bonded V=O group[27,28].

A peroxo group bonded in a side-on fashion to V(V) center, exhibits strong ν(O–O) band at *c.* 870 cm^{-1} and ν_2 and ν_3, which involve metal-oxygen stretches[28], appearing in the region 500–600 cm^{-1}. In the spectra of the complexes **5.1-5.4** in addition to the strong ν(O–O) absorption appearing at *c.*835 cm^{-1}, an additional weak intensity but well resolved band has been observed at a lower frequency range of 810-805 cm^{-1} which has been assigned to the ν(O–O) band of the bridging peroxo group. This may be interpreted as an indication of the presence of two structurally different peroxo groups, the terminal chelated and bridging type. Similar observations were made earlier for compounds wherein the side-on bound and the bridging type of peroxo groups were encountered simultaneously[15,16,29]. Synthesis and structural characterization of a complex, $[F(O_2)\{VO(O_2)F\}_2]^{3-}$, was reported earlier by Schwendt

et al.[13,14] having a bridging peroxo moiety bonded in a μ-η^2: η^2 fashion. IR spectra of these complexes displayed ν(O–O) stretch at a relatively higher frequency of *c.*900 and *c.*870 cm^{-1} indicating that the mode of peroxide co-ordination is probably different in these complexes from that in dinuclear peroxovanadates reported herein and in some previous studies[15,16]. The bands observed at *c.*540 and *c.*620 cm^{-1} were assigned to ν_2 and ν_3 modes of V-O_2 vibrations.

LR (Laser Raman) spectra of the complexes (*Fig. 5.2, 5.4, 5.6* and Table 5.2) complimented their IR spectra confirming the presence of two types of peroxo groups, terminal and bridging peroxides. The bridging and terminally bonded peroxo groups can generally be distinguished on comparing their IR and LR spectra. The ν(O–O) vibration of bridging peroxo group possesses weak dipole and, therefore, shows weak band in the IR spectra but appears strongly in the LR[30] spectra. Appearance of two bands for ν(O–O) at *c.*830 and *c.*805 cm^{-1} in the LR spectra made clear indication of the presence of two structurally different peroxo groups, terminal and bridging peroxides. The bands at *c.*630 and *c.*550 cm^{-1} in LR spectra were assigned to ν_2 and ν_3 modes of V-O_2 vibrations. In the spectra of the complexes the band at *c.*805 cm^{-1}, assigned to ν(O–O), was of weak intensity in the IR but appeared strongly in the LR. This significant observation confirmed the presence of bridging peroxo group in these compounds.

IR spectroscopy is immensely useful and informative for the characterization of heteroligand peroxovanadate 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.

In the spectra of the compounds (**5.1-5.3**) the pattern originating from coordinating peptide showed N-H stretching bands at 3300-3100 cm^{-1} region as expected from the $-N^{+}H_3$ group. The spectra exhibited two distinct bands for the compounds 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[31-33]. 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[34,35]. The ν_s(COO) vibration of the free ligands were observed in the range of 1410–1400 cm^{-1} in the IR spectra[31]. A medium intensity band with some broadening observed in the range of 1350–1300 cm^{-1} region was assigned to ν_s(COO) of the unidentate carboxylate group[31] ($\nu_{as}-\nu_s \approx 300$ cm^{-1}). 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[33]. Appearance of another distinct band at 1395–1405 cm^{-1} in the spectra of the complexes indicated the presence of carboxylate group, presumably coordinated in a bridging bidentate fashion[31] ($\nu_{as}-\nu_s \approx 200$ cm^{-1}). N–H deformation modes (1520-1600 cm^{-1}) in their position and pattern in the spectra of the complexes

Table 5.2. Structurally significant Infrared, Raman and ultraviolet spectral data of peroxovanadate complexes **5.1-5.4**

No.	Compound	Infrared (IR) and Raman (LR) bands, cm^{-1}				UV peak	
		$\nu_s(V-O_2)$	$\nu_{as}(V-O_2)$	$\nu(O-O)$	$\nu(V=O)$	nm	A(1mM)
5.1	$[V_2O_2(O_2)_3(gly\text{-}gly)_3].H_2O$	(IR) 561	613	835m 803w	958s	326	0.35
		(LR) 550	595	835s 805w	955		
5.2	$[V_2O_2(O_2)_3(gly\text{-}ala)_3].H_2O$	(IR) 572	620	835m 803w	949s	310	0.72
		(LR) 560	630	840 810w	960		
5.3	$[V_2O_2(O_2)_3(gly\text{-}asn)_3].H_2O$	(IR) 578	642	815m 798w	930s	322	0.40
		(LR) 580	630	820 805w	942		
5.4	$[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2].H_2O$	(IR) 560	619	845 805	952	328	0.72

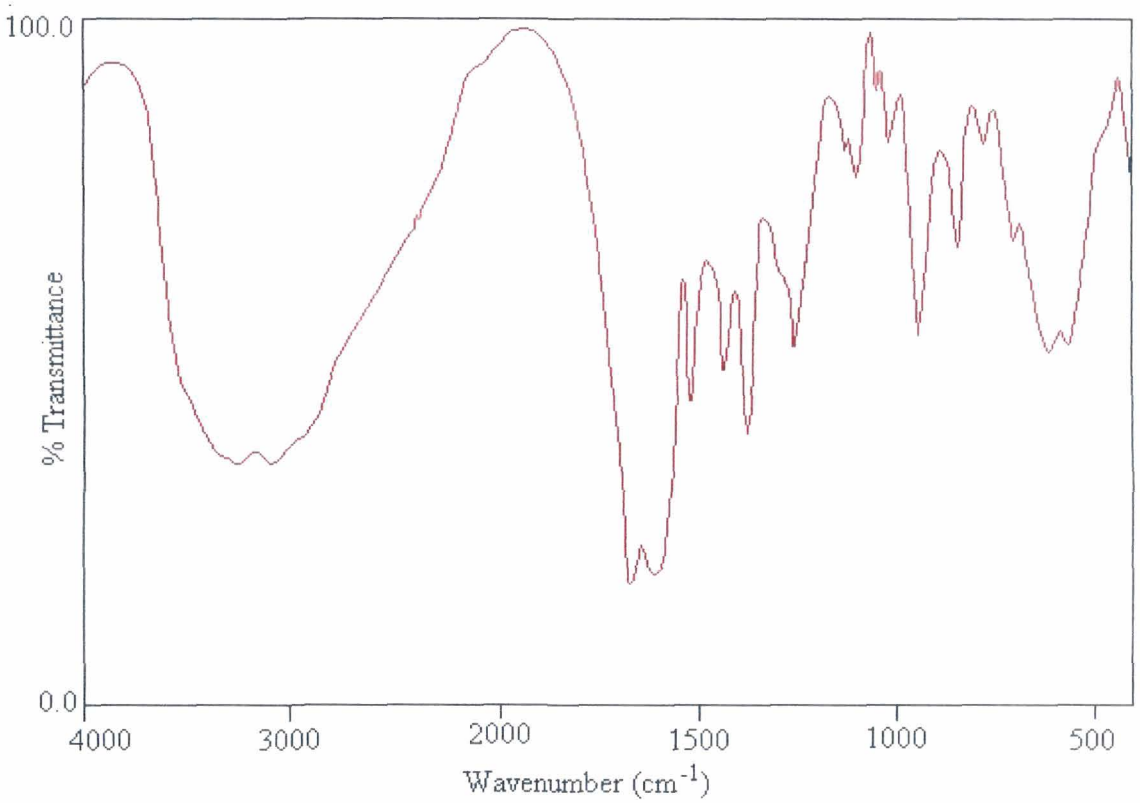

Fig. 5.1. IR spectrum of $[V_2O_2(O_2)_3(\text{ gly-gly})_3].H_2O$.

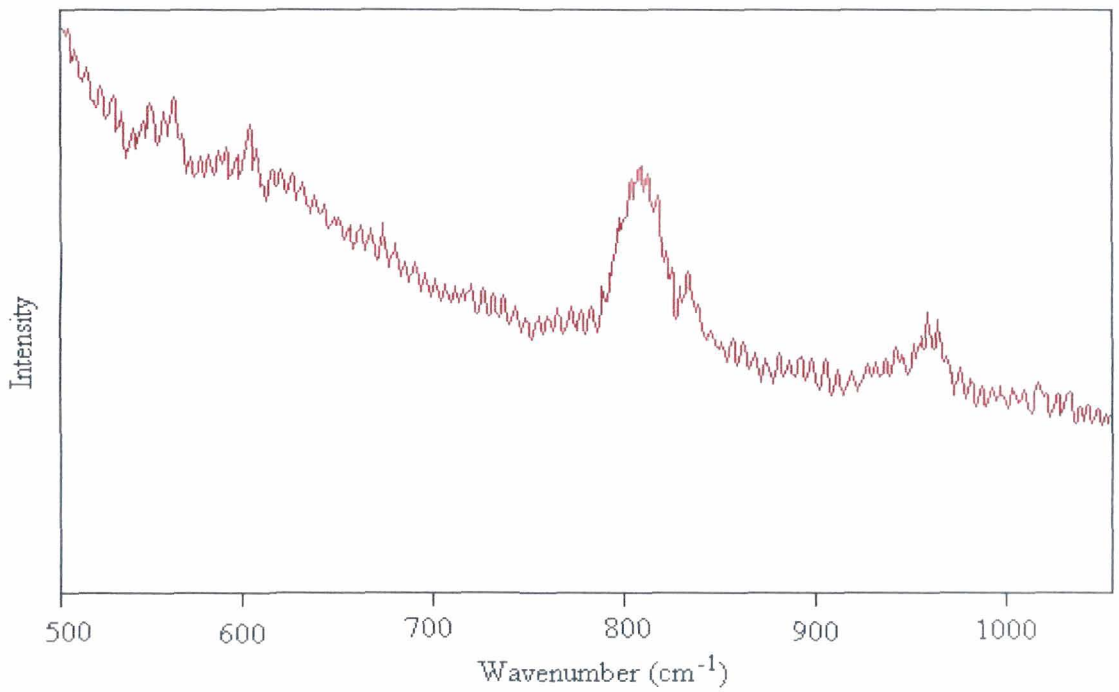

Fig. 5.2. Laser Raman spectrum of $[V_2O_2(O_2)_3(\text{ gly-gly})_3].H_2O$.

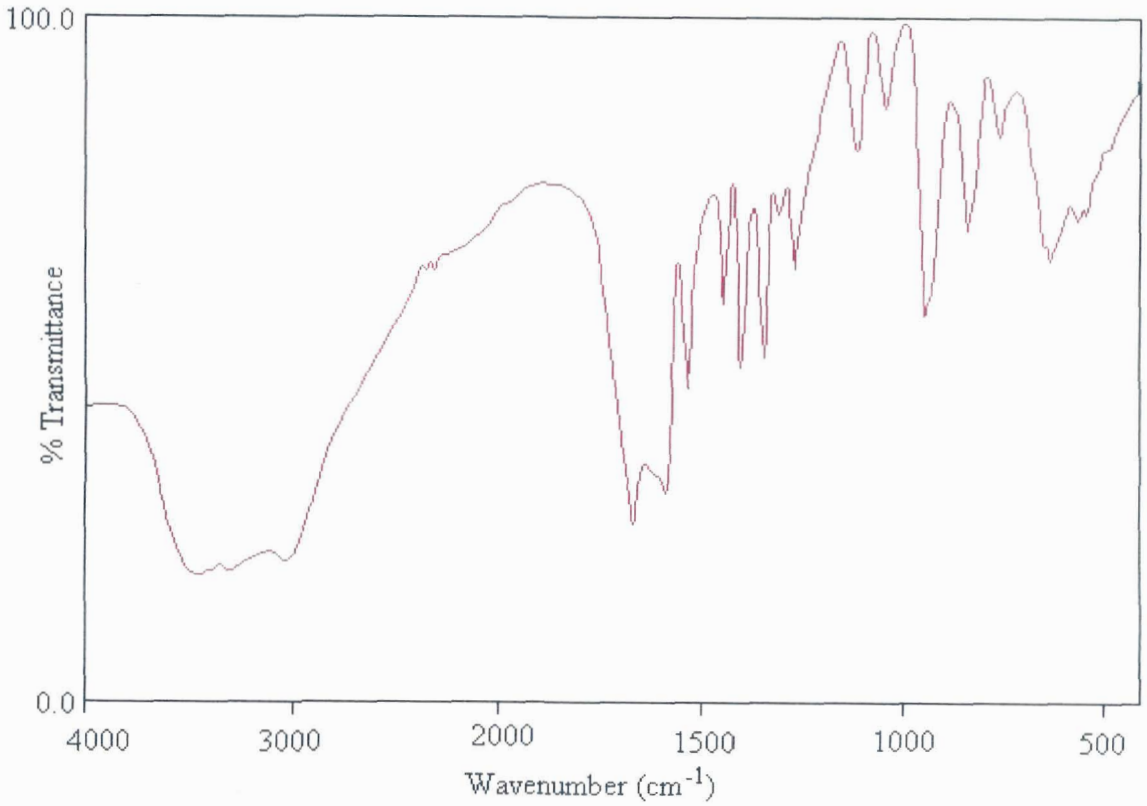

Fig. 5.3 IR spectrum of $[V_2O_2(O_2)_3(\text{ gly-ala})_3].H_2O$.

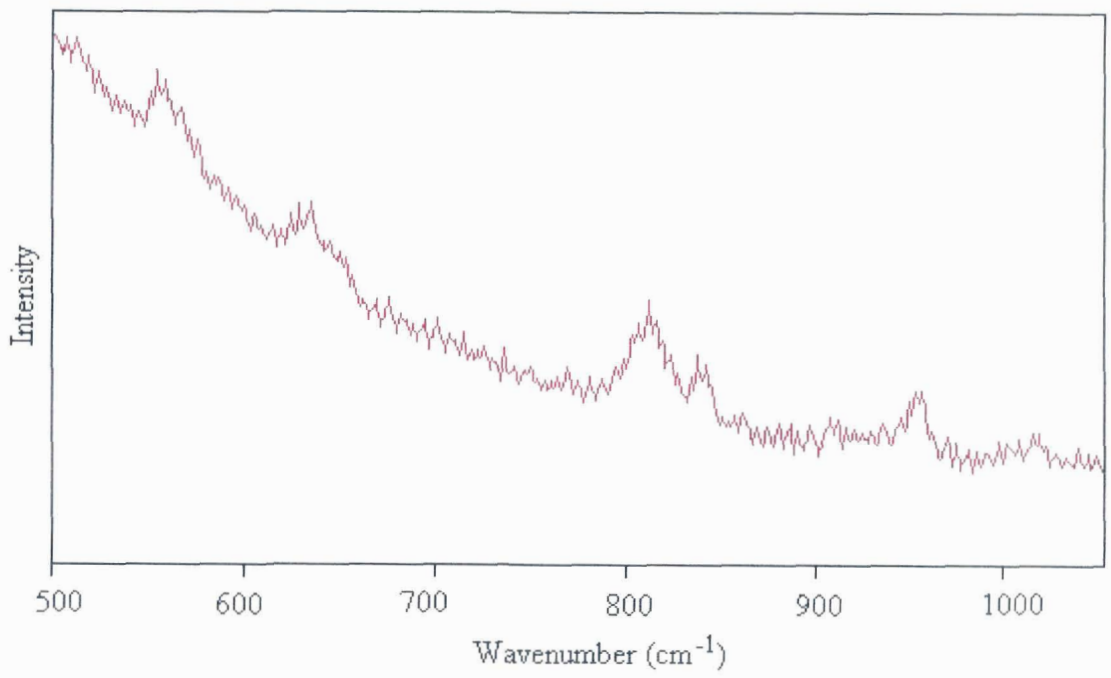

Fig. 5.4 Laser Raman spectrum of $[V_2O_2(O_2)_3(\text{ gly-ala})_3].H_2O$.

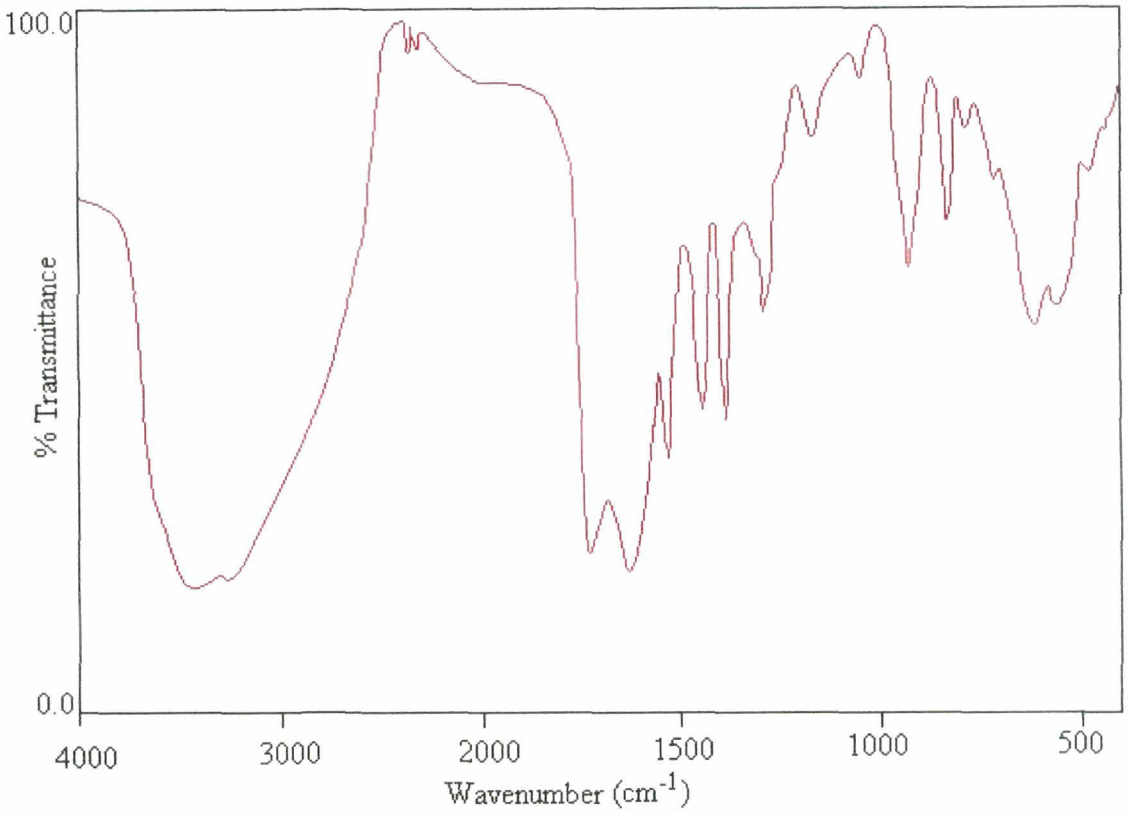

Fig. 5.5 IR spectrum of $[V_2O_2(O_2)_3(\text{ gly-asn})_3].H_2O$.

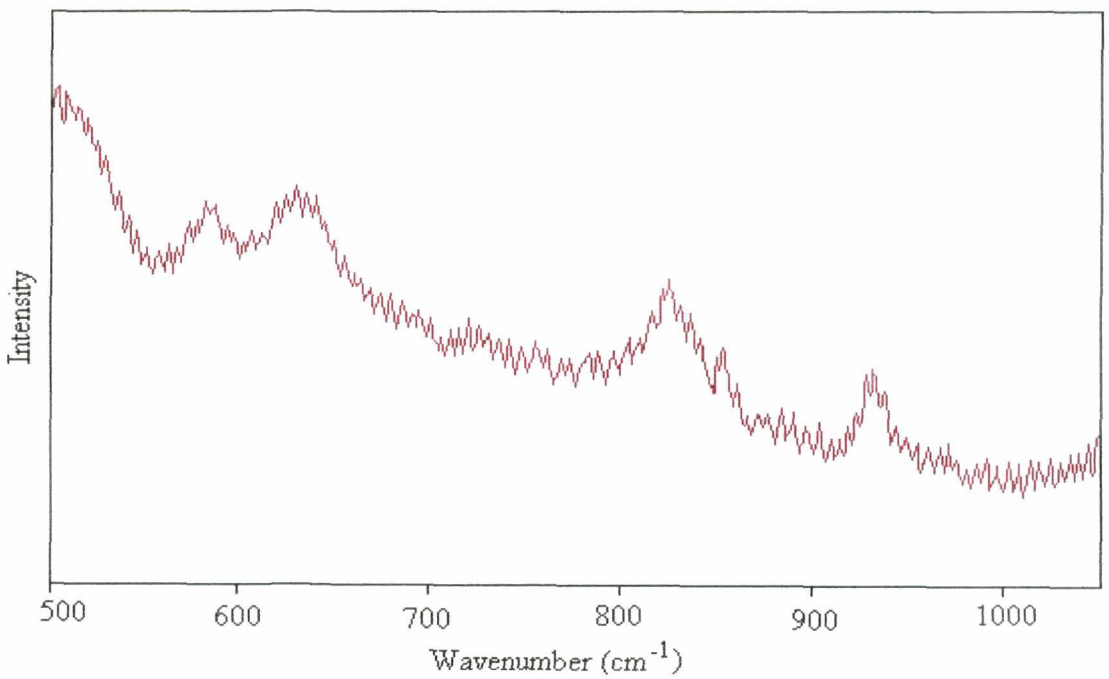

Fig. 5.6. Laser Raman spectrum of $[V_2O_2(O_2)_3(\text{ gly-asn})_3].H_2O$.

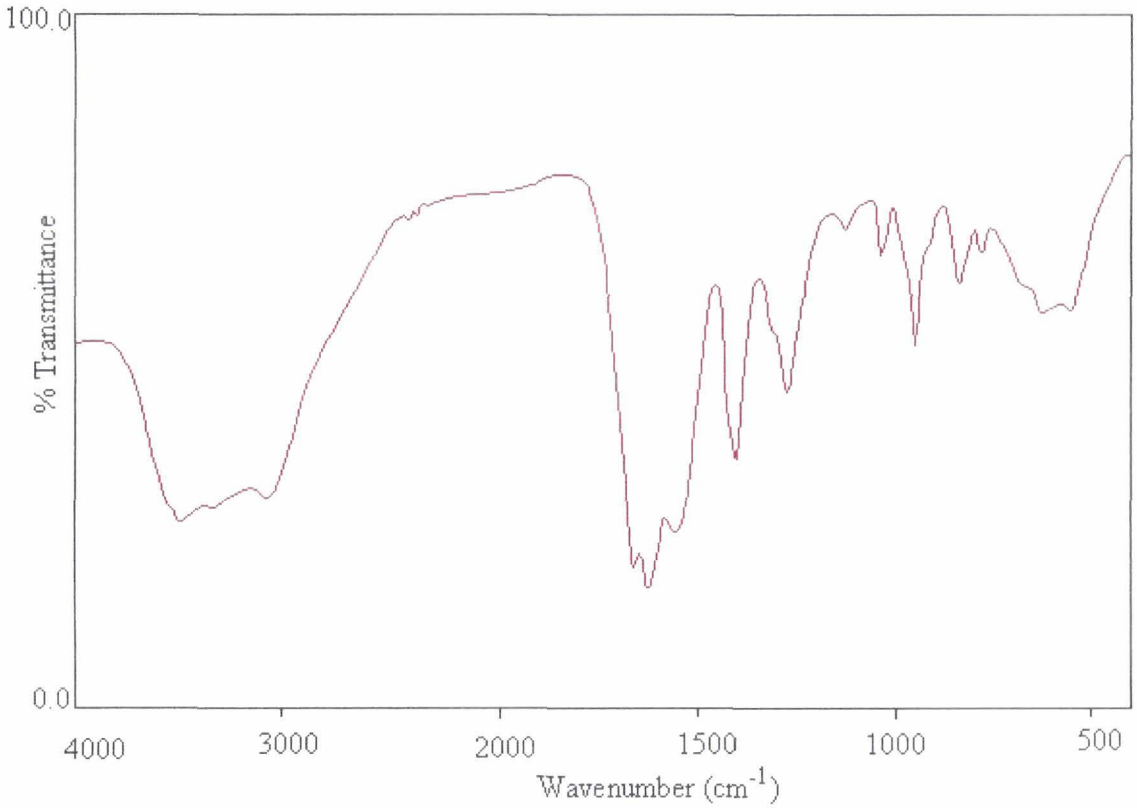

Fig. 5.7 IR spectrum of $[V_2O_2(O_2)_3(\text{ gly-gly-gly})_2].H_2O$.

significantly remained unaltered compared to the free ligand. Observance of a broad band in the vicinity of 3500 cm^{-1} reflects the presence of lattice water.

The strong absorptions appearing in the IR spectrum of complex **5.4**, at 1678 cm^{-1} and 1610 cm^{-1} were assigned to ν(C=O) (amide I) and ν_{as}(COO) modes of co-ordinated triglycine, respectively[31-33]. Unlike the spectra of copounds **5.1-5.3**, in which two bands of comparable intensity attributable to symmetric stretching modes of carboxylate group were clearly resolved in the 1400-1360 cm^{-1} range, for complex **5.4** one distinct band was observed in the spectrum at 1380 cm^{-1} which was assigned to ν_s(COO) mode of unidentate carboxylate group[31]. The broadening of the ν(C=O) signal

with a shift from its free ligand value of *c*.1684 cm^{-1} was probably owing to the presence of both coordinated and free amide groups in the complex, as well as likely participation of free amide groups in hydrogen bonding. Other characteristic of the spectrum was the indication of the presence of lattice water. The spectrum showed N-H stretching bands of coordinated peptide residue in the region of *c*.3255 to 3083 cm^{-1}. The N-H deformation modes of amide and $-N^{+}H_3$ occurred in the region of 1600-1520 cm^{-1}.

Based on these observations it may be inferred that the dipeptide ligands, occurring as zwitterions in the complexes, co-ordinate to vanadium(V) through carboxylate group. Hydrogen bonding between the side chains might stabilize the molecule in the solid state. The structure of these compounds must incorporate the features V: peroxide: dipeptide = 2:3:3, a μ-peroxo group, terminal peroxides, V=O groups, and carboxylate co-ordination of the ligands with vanadium atoms. The proposed structures applicable to the three complexes are shown schematically in *Fig. 5.8*.

Structure envisaged for complex **5.4** is presented in *Fig. 5.9*. The triglycine ligands occurring as zwitterion co-ordinate to the V(V) through O(carboxylate). Co-ordination of one of the carbonyl (amide) groups of the peptide chain probably completes the hexa co-ordination of vanadium in the complex leading to the formation of a seven membered ring around each vanadium. The second amide group in the peptide side chain is not shown in the structure for simplicity. It is possible that hydrogen bonding between the peptide side chains stabilize the molecule.

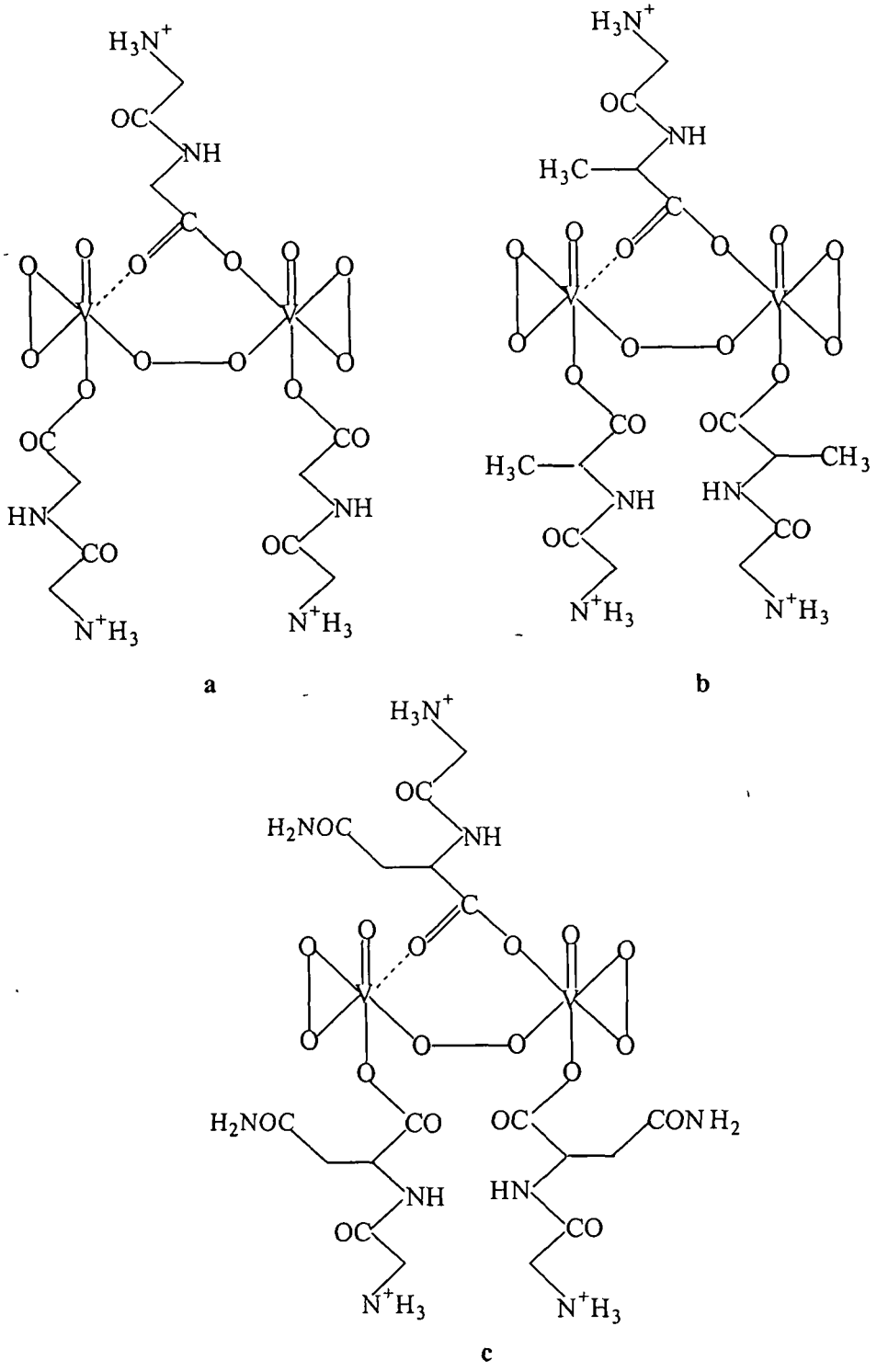

Fig. 5.8. A representation of proposed structures of peroxovanadate-dipeptide complexes. (**a**) $[V_2O_2(O_2)_3(gly\text{-}gly)_3].H_2O$; (**b**) $[V_2O_2(O_2)_3(gly\text{-}ala)_3].H_2O$; (**c**) $[V_2O_2(O_2)_3(gly\text{-}asn)_3].H_2O$.

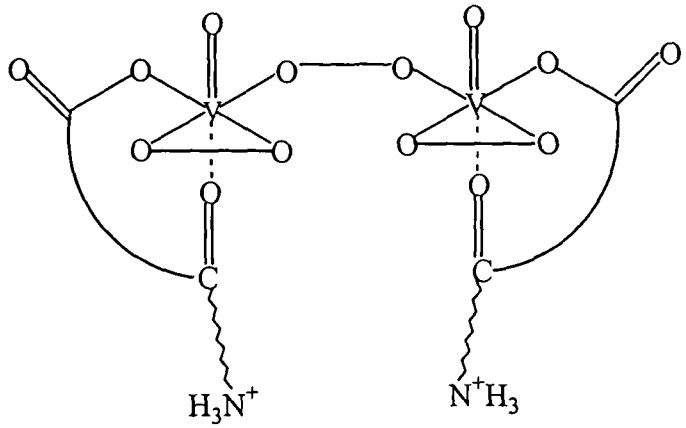

Fig. 5.9. Proposed structure of peroxovanadate-tripeptide complex $[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2].H_2O$. The second amide group of the peptide side chain is not shown.

5.3.2 Release of oxygen from the peroxovanadium compounds - action with the enzyme catalase

On adding solids of these compounds to water (*c*.2 mg/ml), bubbles of gas came out of solutions for a few min. Oxygen was confirmed to be the gas released at exceedingly high rates of about 50 μM/min for short periods by which time the bubbles formed interfered with the measurement in the oxygraph. Instability of the complexes in water and degradative loss of peroxide groups were thus implicit.

The electronic spectra of the compounds **5.1-5.4** in aqueous solution recorded after bubbles ceased (after 30 min) exhibited a weak intensity broad LMCT band originating from peroxide to vanadium $\pi^*-d\sigma$ transition[15] at 310-330 nm (Table 5.2, *Fig. 5.10*). Variations in absorbance values indicated partial loss of peroxide on making solutions of these compounds. The intensity of this band was found to decrease with

time consistent with the loss of peroxide from these complexes and their unstable nature.

In order to further explore the nature of the species remaining in solution after oxygen evolution ceased to appear we were interested to study their activity with the enzyme catalse. On addition of catalase to the solutions of **5.1**, **5.3** and **5.4** after oxygen evolution ceased, further slow release of oxygen was recorded (Table 5.3). This indicated that the products in such solutions were indeed peroxovanadates and is expected to be diperoxovanadate species at pH 7.0. Under this condition the reaction of catalase with H_2O_2 will be completed in less than 2 min. This slow release of oxygen from peroxo groups by catalase is typical of DPV, and is dependent on concentration of DPV and catalase[36]. A maximum of 1.5 O_2 per mole of the compound will be released if all the three peroxides were retained. Experimental lower values of 0.7-0.2 O_2 per mole of the compound were realized since part of the peroxide was initially lost during the process of solution preparation. The subsequent secondary rates of oxygen release, calculated from the data, paralleled the residual peroxide concentrations. Addition of EDTA (1mM) to such solution decreased the rate of oxygen release to about 15% indicating resistance of their EDTA complex to catalase action.

5.3.3 ^{51}V-NMR spectral analysis of aqueous solution of the complexes **5.1 - 5.4**

Further information regarding the nature of the complexes in solution was derived from ^{51}V-NMR studies (*Fig.5.11* and *5.12*). The assignment of the peaks in the present study was on the basis of available data[17,18,37,38]. The solutions of compounds being acidic, it may be expected that free vanadate will be formed on depletion of its peroxide and will oligomerize to decameric form (V_{10}). The spectra of the complexes

Table 5.3. *Catalase dependent oxygen release from peroxovanadate complexes*

No.	Compound	Conc. mM	Oxygen release		ΔO_2/compound (mol ratio)
			μM/min	Total, μM (ΔO_2)	
5.1	$[V_2O_2(O_2)_3(gly\text{-}gly)_3].H_2O$	0.2	12.3	134	0.67
5.3	$[V_2O_2(O_2)_3(gly\text{-}asn)_3].H_2O$	0.2	5.0	46	0.23
5.4	$[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2].H_2O$	0.2	12.1	130	0.60
	DPV	0.2	7.0	96	0.48

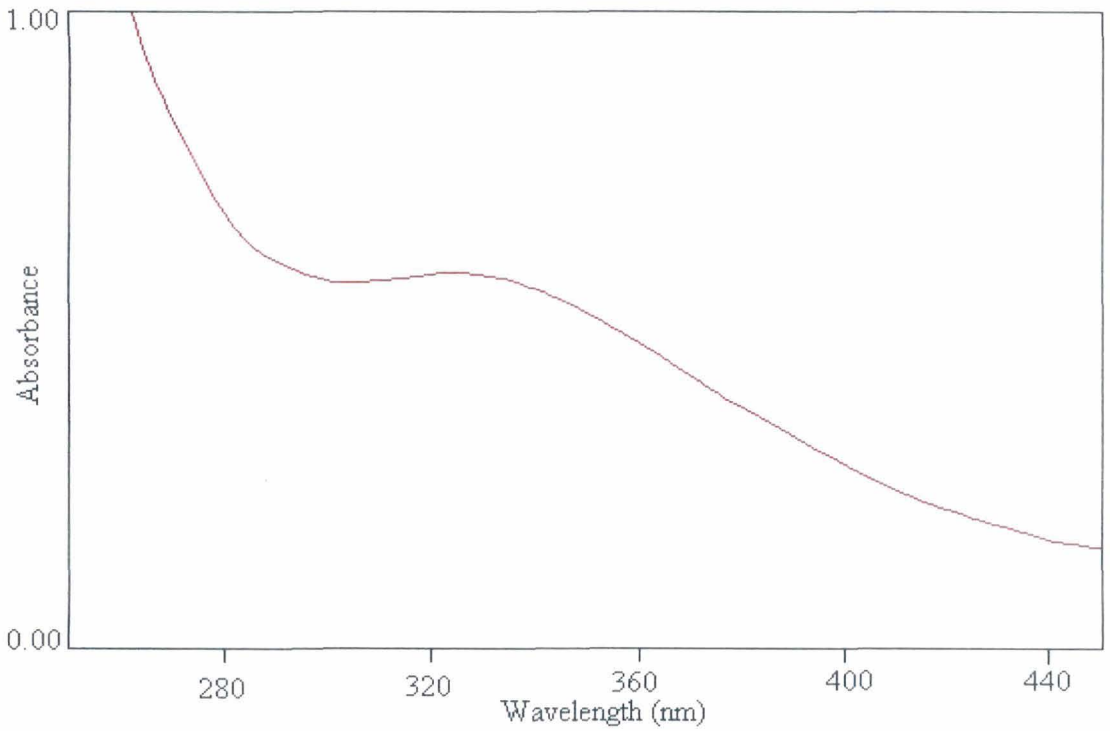

Fig. 5.10 UV spectrum of $[V_2O_2(O_2)_3(gly\text{-}gly)_3].H_2O$.

displayed three peaks at -427, -509 and -527 ppm with intensity ratio of 1:2:2 which have been assigned to V_{10}. The major signal at -694 ppm indicated the presence of diperoxovanadate as the predominant species. The small variations from the reported chemical shift values may be due to the presence of the co-ordinated ligands in some of the products and to variation of pH. The less intense peaks observed at -545 and -650 ppm were assigned to V_1 and residual MPV possibly retaining the peptide ligand. The two weak resonances appearing near -714 ppm were probably due to the presence of diperoxovanadate species containing peptide ligand co-ordinated through carboxylate group[17,18]. Thus from the NMR spectral studies it was further apparent that peroxo-bridged dimeric complexes undergo rapid degradation in water and hence provide no direct information regarding nature of the original solid compound.

5.4 DISCUSSION

The importance of pH for the successful synthesis of peroxo-metal compounds has been emphasized in the literature[2,37-40]. Since our primary concern was to isolate complex with a $(VO)_2(O_2)_3$ moiety which has been reported to exist in acidic solution[6], the pH value of *c.*2 attained spontaneously during the reaction was not raised.

Preferred mode of co-ordination of an amino acid or a peptide is also dependent on pH of the reaction medium and the type of the metal[17-19,35,41-44]. Depending on the reaction conditions a peptide ligand provides several alternative co-ordination sites to the metal, viz., terminal amino and carboxylate groups as well as the amide groups of the side chain and hence can act as mono, bi, or tridentate ligand with different combinations of donor atoms and can occur in complexes in either neutral zwitterionic

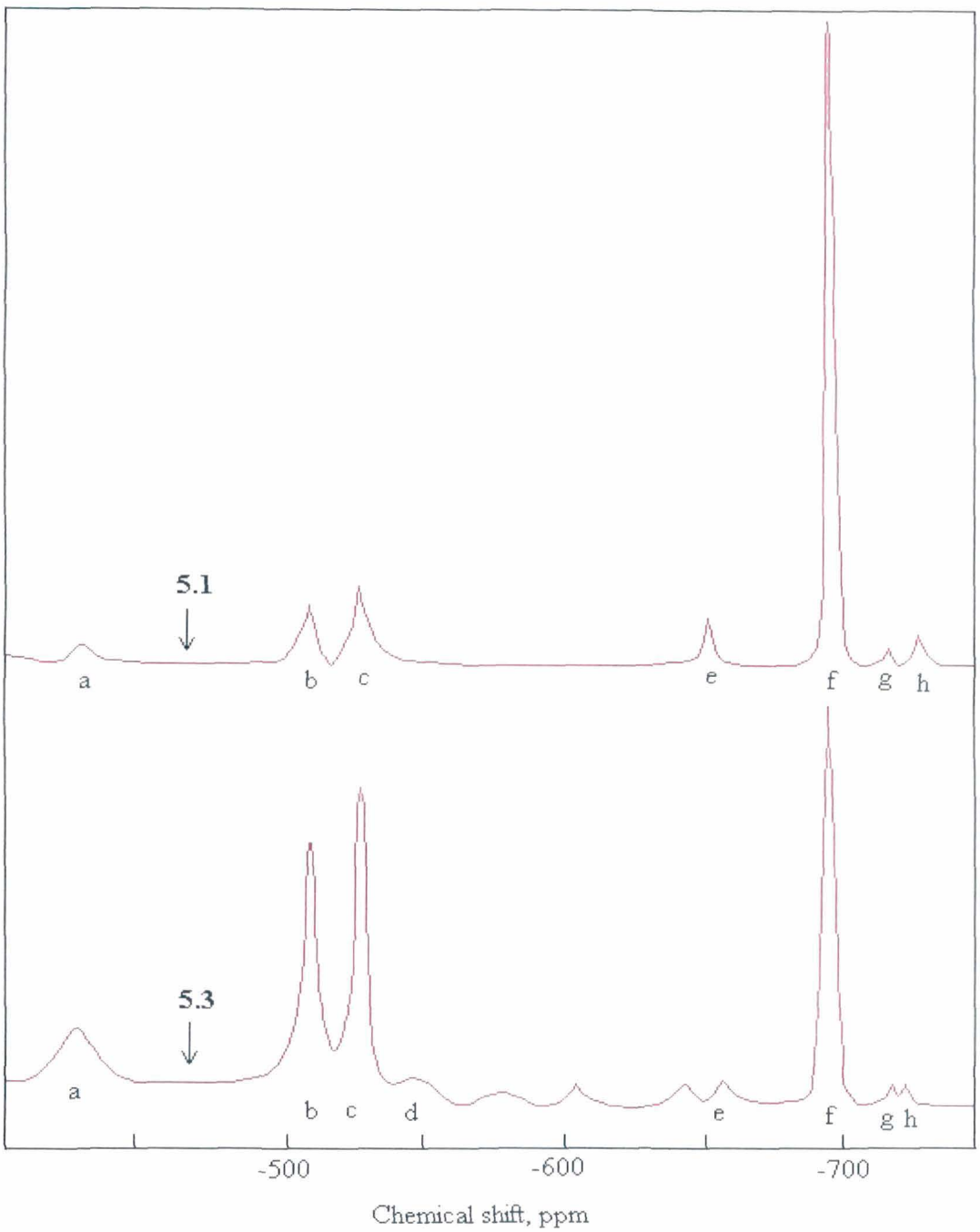

Fig. 5.11. ^{51}V-NMR spectra of aqueous solutions of peroxovanadate-dipeptide complexes **5.1** and **5.3.** Solutions of the compounds were obtained by adding the solids to water and waiting untill the bubbles ceased. Identification of the peaks **:** a, b and c, the three peaks (2:2:1) of decavanadate (V_{10}); d, free vanadate (V_1); e, liganded monoperoxo-vanadate(MPV); f, diperoxovanadate (DPV); g and h liganded (DPV).

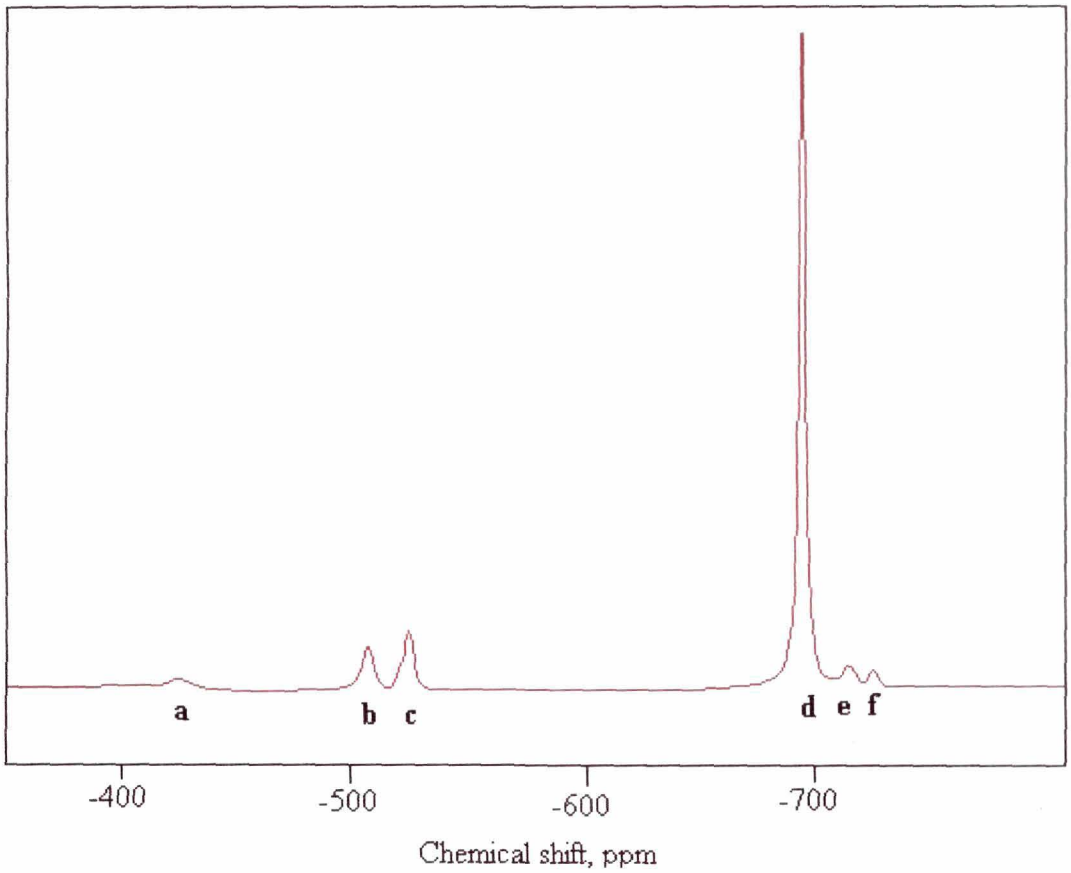

Fig. 5.12. ^{51}V-NMR spectra of aqueous solution of peroxovanadate-tripeptide complex. Solution of the compound was obtained as described under *Fig. 5.11*. Identification of the peaks : a, b and c, the three peaks (2:2:1) of decavanadate (V_{10}); d, diperoxovanadate (DPV); e and f liganded (DPV).

form[41,42] or the anionic form[22,35,41-43,45]. In addition, they are known to form bridge between metal atoms leading to the formation of dinuclear or polynuclear structures[46]. Thus bonding of more than one amino acid or a simple peptide to the same metal center in different co-ordination modes as observed in the case of complexes **5.1-5.4** may appear unusual, however, is not unprecedented[41,44,46]. Tridentate co-ordination of

gly-gly involving carboxylate, deprotonated N-amide and -NH_2 groups in a structurally characterized monoperoxo-vanadate compound, was reported previously[22]. This compound was isolated from a solution of pH 6.8. In the present case, the low pH of the reaction medium probably favored the co-ordination of the di- and tripeptide ligands to V(V) in their zwitterionic form through carboxylate group thereby stabilizing the peroxo-bridged divanadate moiety and leading to the synthesis of the desired molecular complexes. In case of compound **5.4**, it is plausible that the two coordinated triglycine ligands in the coordination sphere, because of their relatively longer chain length, prefer to act as bidentate ligand thereby completing hexa-coordination around each vanadium instead of allowing bridge formation by a third triglycine molecule[41]. The glycyl-peptides with hydrophobic amino acids, valine and leucine failed to give a solid product under similar conditions which suggest that interaction of polar side chain may have a role in stabilizing the product.

The stabilization provided by the peptide co-ligands to the μ-peroxovanadate moiety formed in solution, affording their isolation into solid state, would presumably be lost in aqueous solution resulting in the break up of the VOOV group with subsequent release of oxygen. The peroxo groups which are retained in the degradation products and are responsible for the further release of oxygen under the effect of catalase action, are the ones bonded in a side-on fashion as in DPV. Presence of DPV in solution of the compound has been confirmed from their ^{51}V-NMR and electronic spectra.

In summary, we have demonstrated that it is possible to isolate the dioxo-triperoxo vanadate species formed in solution into solid state, through complexation

with small peptides, under appropriate experimental conditions. The occurrence of the peptide ligands as zwitterions and their possible involvement in hydrogen bonding between the side chains permit the isolation of the title compounds into solid state as neutral molecular complexes. The compounds possess unique features which may be of interest for a deeper understanding of the peroxovanadium(V) chemistry. Redox properties of the newly synthesized compounds **5.1-5.4** are represented in Chapter 6 of the thesis.

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CHAPTER 6

Peroxo-bridged Vanadium(V) Complexes as Selective Bromide Oxidant in Bromoperoxidation

Peroxo-bridged Vanadium(V) Complexes as Selective Bromide Oxidant in Bromoperoxidation*

6.1 INTRODUCTION

Bromoperoxidase, involved in the biosynthesis of a variety of brominated natural products, is the first enzyme shown by ESR[1] and NMR[2] studies to contain protein bound vanadium that is essential for its activity. By itself H_2O_2 is capable of oxidation of bromide in acid medium[3] but is ineffective in physiological conditions 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[4]. 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[1] although, its exact speciation is still a matter of speculation.

The choice of vanadium in these enzymes[1,2] and its known ability to form complexes[5-8] with H_2O_2 led to implication of peroxovanadate as the active bromide oxidant. The H_2O_2-dependent oxidation of bromide is a two-electron transfer both in the enzyme reaction[9] at pH 6.5 and in the chemical reaction with cis-dioxovanadium in highly acidic medium[3]. Vanadium atoms appear to aid the overall catalytic process by presenting a modified peroxide species as the oxidant at physiological pH. The biomimetic models of bromoperoxidation reaction therefore proposed vanadium

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derivatives such as peroxovanadates[10-12] and their oxo or peroxo-bridged dimers[13-15] as the oxidants. The credit for first biomimetic functional model of bromoperoxidase goes to Sakurai and Tsuchia[10]. They found bromination of an acceptor occurred in phosphate buffered medium (pH 6.0) containing excess H_2O_2 and KBr in presence vanadyl sulfate, but not vanadate. Another fully functional mimic of the enzyme, cis-dioxovanadium(V) (VO_2^+) in acidic aqueous solution, was reported by Butler and co-workers which was shown to catalyze bromination of 1,3,5-trimethoxybenzene (TMB) as well as bromide-assisted disproportionation[3] of H_2O_2. About the same time Bhattacharjee reported that a mixture of V_2O_5 and H_2O_2 was effective in bromination of organic substrates[12].

The mono- and diperoxovanadate species (MPV and DPV) are readily formed on adding excess H_2O_2 to vanadate solution[7,8] with DPV predominating at pH > 5.0. Initially both these species were proposed to be bromide oxidants based on the ^{51}V-NMR evidence of their occurrence and changes during the progress of the bromination reaction[9,11]. However, synthetic DPV and compounds containing these peroxovanadate moieties could not substitute for V_2O_5 and H_2O_2 mixture in bromination[13]. Clague and Butler later observed that the rate of bromide oxidation was second order in vanadium concentration, and was maximal when MPV and DPV were equal in concentration[3]. A dimeric peroxovanadate species $(VO)_2(O_2)_3$ presumed to be formed by a combination of $VO(O_2)^+$ and $VO(O_2)_2^-$ was then proposed to be the critical oxidant of bromide[3] however, this species was found only in highly acidic medium[3,16] and with high concentrations of vanadate and H_2O_2. Also, how this species gains oxidant activity is not clear although possible involvement of a bridging peroxide has been proposed[15].

Studies on synthetic peroxovanadate complexes as functional and structural models of bromoperoxidase[3,12,15,17-19] 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[3,12,15,17-21]. In recent years several vanadium complexes of multidentate ligands containing O and N donor sites were tested for catalysis of bromide oxidation in presence of hydrogen peroxide[18-20,22]. Contrary to natural V-BrPO which is most efficient at pH 5.5-7 several model complexes were found to be catalytically active in acid medium[17-20].

It was observed by Ramasarma and co-workers that diperoxovanadate, which is inactive in bromide oxidation on its own, acquires oxidant activity in presence of vanadyl or vanadate[14] at pH >5. Either uncomplexed vanadate or vanadyl was required for bromination by DPV, and this has been substantiated by demonstration of remarkable enhancement in rates of bromination on their addition to the system containing DPV alone[14]. These findings led to the proposal that μ-peroxo-divanadate intermediate, $[OVOOV(O_2)]^{3+}$ formed by complexation of these two species is the proximate oxidant of bromide[14] at physiological pH. Support for such an intermediate as the bromide oxidant came from the studies on a synthetic compound with a VOOV bridge, $[V_2O_2(O_2)_3(Gly)_2(H_2O)_2]$, which was found to be highly active in oxidizing bromide at physiological pH, and liberating bromine gas in the absence of an acceptor[23]. Besides bromide oxidation, as mentioned in Chapter 3, other reactions also occur when an oxidisable substrate is present during interaction of vanadyl and DPV. These are oxidation of NADH[24], inactivation of glucose oxidase[25], and hydroxylation of benzoate[26]. Oxygen is released in absence of a substrate[26].

Thus it is evident that exact mechanism involved in the function of bromoperoxidase and the actual role of vanadium in these processes are yet to be understood completely despite the progress made in recent years. Further insight into

actions of protein-bound vanadium needs more information on structure, bonding and reactivity of peroxovanadate compounds with bio-relevant ligand environment. Since the newly synthesized peroxo-bridged compounds **5.1-5.4** were closely related to the proposed peroxobridged intermediate mentioned above, we focused on investigating their activity in peroxidative bromination reaction. The objective of our study was to find an oxidant of bromide with good activity at physiological pH, an essential requirement of biomimetic model.

The compounds **5.1-5.4** instantaneously oxidized bromide to a bromination competent intermediate at pH>5. Chapter 6 of the thesis presents an account of the reactivity the complexes $[V_2O_2(O_2)_3(dipeptide)_3].H_2O$ [dipeptide = glycyl-glycine (**5.1**), glycyl-alanine (**5.2**), or glycyl-asparagine (**5.3**)] and $[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2].H_2O$ (**5.4**) in oxidative bromination. Selective activity of the peroxo-bridged divanadate for bromide oxidation is indicated by their inability to support oxidation of NADH and inactivation of glucose oxidase.

6.2 EXPERIMENTAL SECTION

6.2. 1 Measurement of bromination activity in solution

The method of de Boer et al.[27] described in Chapter 2, was used to measure bromination activity of the synthesized peroxovanadium compounds **5.1-5.4** in solution. 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 (2 M) and phenol red (20 μM). The redox activity was tested by adding weighed amount of the solid compounds to the reaction mixture, and by

monitoring possible change in absorbance at 592 nm at 30° C. The volume of the reaction mixture was kept at 25 ml to enable accurate weighing of small amounts of solid samples of peroxovanadates added. Aliquots were transferred to the spectrophotometer immediately after mixing to record the jump in A_{592}. The added bridging-peroxo complexes acted directly as bromide oxidant producing instant change in A_{592} and therefore represented as 'instant activity'. The steady rate of increase that followed due to residual peroxovanadate is referred to as 'secondary rate'. The data on bromination activity of the compounds are given in Table 6.1.

6.2.2 Measurement of glucose oxidase activity

The reaction mixture contained phosphate buffer (50 mM, pH 7.0), glucose (10 mM), and glucose oxidase (4.6 μg protein/ml) and the reaction was started by adding glucose solution[25]. The consumption of oxygen was followed in an oxygraph, and the activity was expressed as the rate (μM/min). Pretreatment of glucose oxidase (2.3 mg protein in 10 ml) was carried out in phosphate buffer (50 mM, pH 5.5) by adding weighed samples of (1.5-9.0 mg/10ml) solid compounds and incubating for 10 min at 30°C. A suitable aliquot to give 4.6 μg protein/ml in the reaction mixture in the oxygraph was then tested for the enzyme activity by the rate of oxygen consumption. The reagents carried into the glucose oxidase assay medium at this dilution had no effect on the assay.

6.3 RESULTS

6.3.1 Bromination reaction with solid peroxo vanadium(V) compounds

The bromination of phenol red to bromophenol blue was employed to investigate the bromination activity of the complexes **5.1-5.4** in solution. Addition of an weighed amount of solid compound to the standard reaction of bromide in phosphate buffer with phenol red as trap for oxidized bromine resulted in instantaneous colour 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*). After the initial very fast bromination activity referred as "instant" activity, a slow increase in A_{592} indicated a secondary rate of bromination (*Fig.6.2* and *Fig. 6.3*). The data in Table 6.1 show the instant and secondary bromination activities of dinuclear complexes **5.1-5.4**.

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 bridging peroxo compounds (*Fig. 6.4*). 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 (*Fig. 6.4*). 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[1].

The results of the afore mentioned experiments suggest the occurrence of two distinct types of bromination reactions. The fast bromination reaction which give a large increase in A_{592} indicate the presence of an active group in the peroxo-bridged complexes that readily oxidizes bromide. It is evident that the degradation products of the compounds formed in solution are responsible for the slow secondary bromination

reaction. The instant activity of 37-43% recorded, although small, substantiated the oxidant capacity of the original compounds in spite of their being highly unstable in solution.

Under these conditions DPV (1mM) was completely inactive on its own[14], however, in presence of vanadate showed the secondary rate but not the instant activity (Table 6.1).

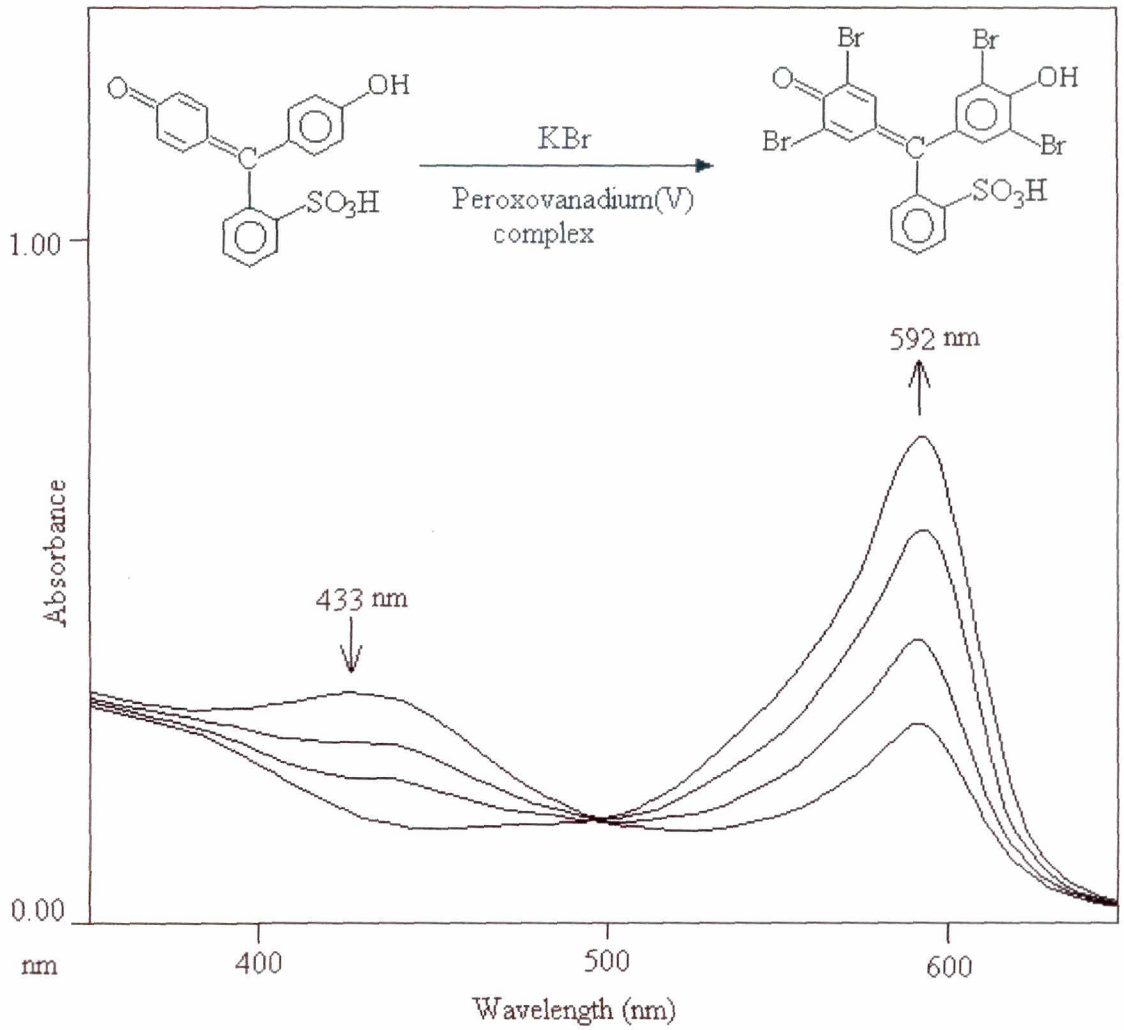

Fig 6.1 Spectral changes at 5 min interval following bromination of phenol red to bromophenol blue on addition of solid complex **5.3**. The reaction mixture contained phosphate buffer (50 mM, pH 5.5), KBr (2M) and phenol red (20 μM).

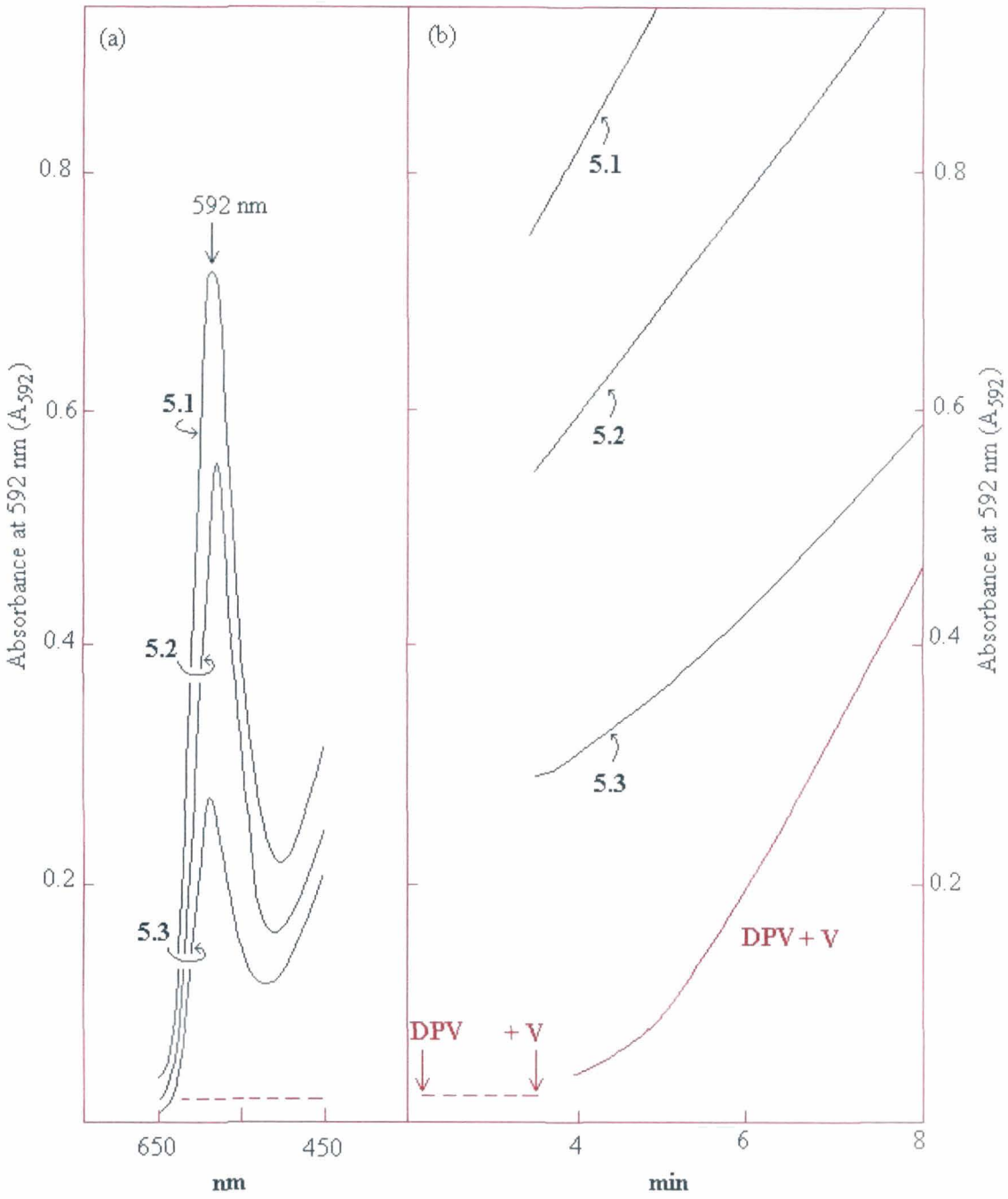

Fig 6.2 Bromination activity with dinuclear peroxovanadate-dipeptide complexes. (**a**) The spectra were recorded immediately after adding the solid compounds to the reaction mixture showing the "instant activity"; (**b**) increase of A_{592} indicating the secondary rate of bromination by the residual peroxovanadates. The numbers of **5.1-5.3** and DPV (+V) on the lines identify the additions.

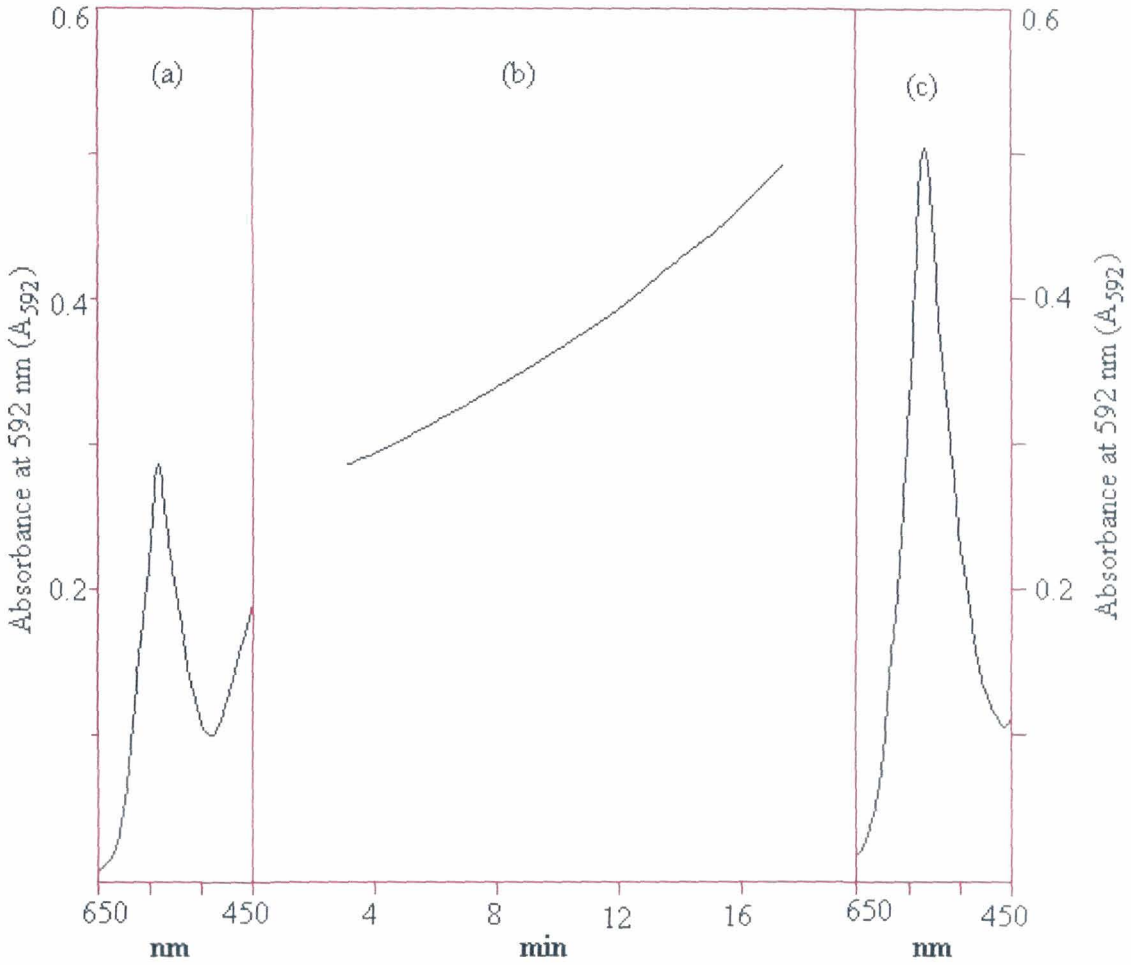

Fig 6.3 Bromination activity with dinuclear peroxovanadate-tripeptide complex (a) spectrum taken immediately after adding the solid compound **5.4** (0.14 mg/ml) to the reaction mixture showing the "instant activity"; (b) the increase of A_{592} indicating the secondary rate; (c) the peak at 592 nm at 18 min of the reaction.

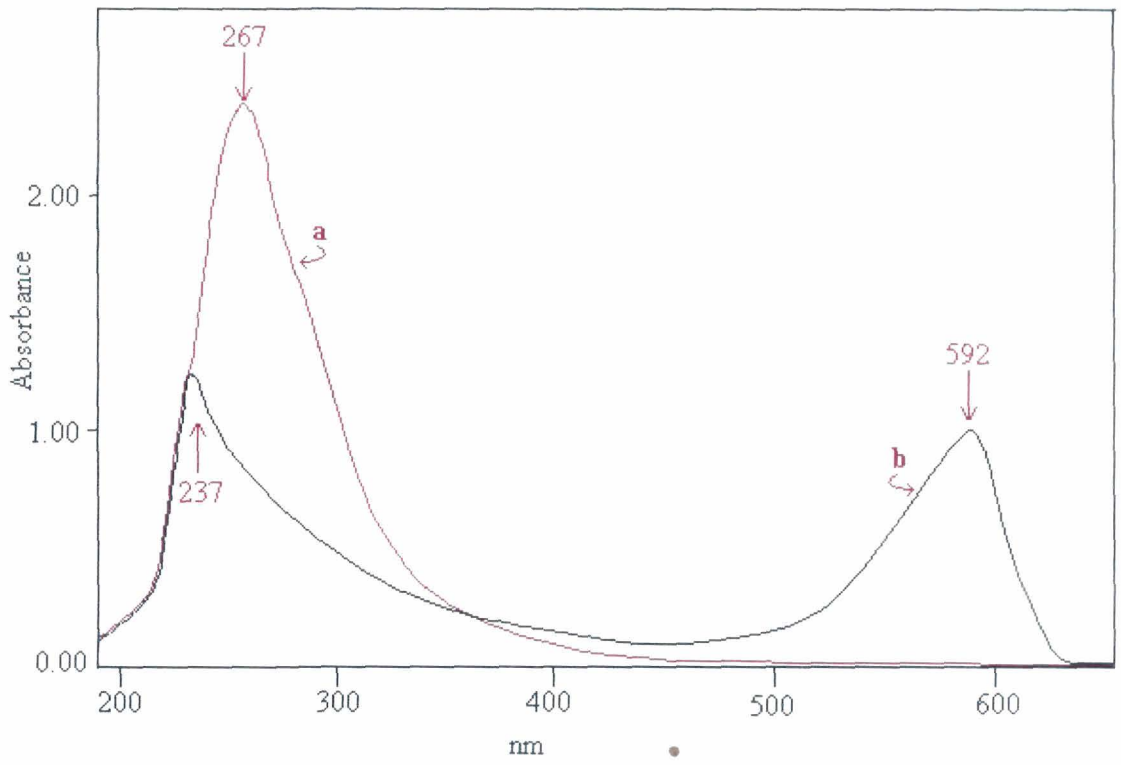

Fig 6.4 Spectral changes following bromination of phenol red to bromophenol blue on addition of solid complex **5.1**. The reaction mixture contained phosphate buffer (50 mM, pH 5.5), KBr (2M) and phenol red (20 μM). (**a**) KBr + compound in absence of phenol red; (**b**) KBr + compound + phenol red.

Table 6.1 *Bromination of phenol red with peroxovanadate complexes*

No. Compound	Conc. mg/ml	mM	Instant activity ΔA_{592}	Instant activity bromine transfer total, μM	Instant activity bromine transfer μM/ mM compd.	Secondary rate (extrapolated to 1 mM compdound) μM Br/min
5.1 $[V_2O_2(O_2)_3(gly\text{-}gly)_3].H_2O$	0.30	0.46	0.77	46	100	17.8
5.2 $[V_2O_2(O_2)_3(gly\text{-}ala)_3]H_2O$	0.29	0.42	0.56	33	79	6.6
5.3 $[V_2O_2(O_2)_3(gly\text{-}asn)_3]H_2O$	0.22	0.27	0.28	17	63	6.5
5.4 $[V_2O_2(O_2)_3(gly\text{-}gly\text{-}gly)_2]H_2O$	0.14	0.22	0.24	14	64	1.6
DPV		1.00	nil	nil	nil	nil
DPV(+vanadate, 0.1 mM)		1.00	nil	nil	nil	4.2

6.3.2 Reaction with solutions of peroxovanadate compounds

Reactions conducted by addition of an aliquot of title compounds from a freshly prepared solution, instead of the solid compound, to the standard reaction mixture gave a good rate of increase in A_{592}. The rate progressively decreased when equal aliquots were tested from this stock solution at 2-4 min interval followed by total loss of activity in about 2 h of making of the solution. These experiments further confirmed that vanadate products derived from the peroxo-bridged complexes, responsible for the secondary slow bromination reaction, also undergo further inactivation.

These rapid inactivation processes constrained our studies on the kinetics of the reaction. Data collected by using a freshly prepared solution for each experiment indicated that increases in rates are nonlinear with increase in concentration of title complexes and KBr. The rate showed a linear relationship when plotted against the square of concentration of bromide indicating a second order dependence with respect to KBr.

6.3.3 Effect of buffer

Omission of phosphate buffer from the reaction medium had no significant effect on the instant bromination activity of the complexes although, a small decrease of about 10% was observed in the secondary rate of bromination. This indicated that the presence of phosphate was not essential for such activity of dinuclear peroxovanadate complexes. This is in contrast to the requirement of phosphate with vanadate-H_2O_2 system as bromide oxidant[14]. Vanadate is known to react with most of the compounds

used in buffers including phosphate[28]. With respect to vanadates Hepes is fairly inert and is the recommended buffer[28,29]. However, in the present study fast bromination activity was found to be suppressed when Hepes (pH 6.5) was used as buffer indicating possible interaction between Hepes and the bimetallic peroxovanadate compounds. In investigations dealing with peroxovanadates, a near neutral phosphate buffer was found to be reasonably inert and its use proved to be satisfactory in several studies[23,29,30]. Instant bromination activity was realized with the compounds **5.1-5.4** in phosphate buffer at pH 7.0. The data in Table 6.1 were obtained at pH 5.5 in order to record the secondary activity.

6.3.4 Inhibition of bromination reaction

Inhibitor studies are valuable in giving clues on the involvement of reactive species. The effect of H_2O_2 and EDTA on bromination reaction under standard assay conditions was tested. The initial fast bromination reaction was inhibited only to the extent of 5-10% with H_2O_2 (10 Mm) and *c.*20% with EDTA (10 mM). After the initial jump in A_{592} the peak intensity remained unchanged with time in contrast to its slow increase in the absence of inhibitors, indicating complete inhibition of the secondary reaction (*Fig. 6.5*). It is thus inferred that the peroxo-bridged dimeric vanadate is probably the most proximate intermediate in bromide oxidation as it is only partly inhibited by H_2O_2 and EDTA which terminate the secondary bromination reaction carried out by degradation products of the dinuclear complex.

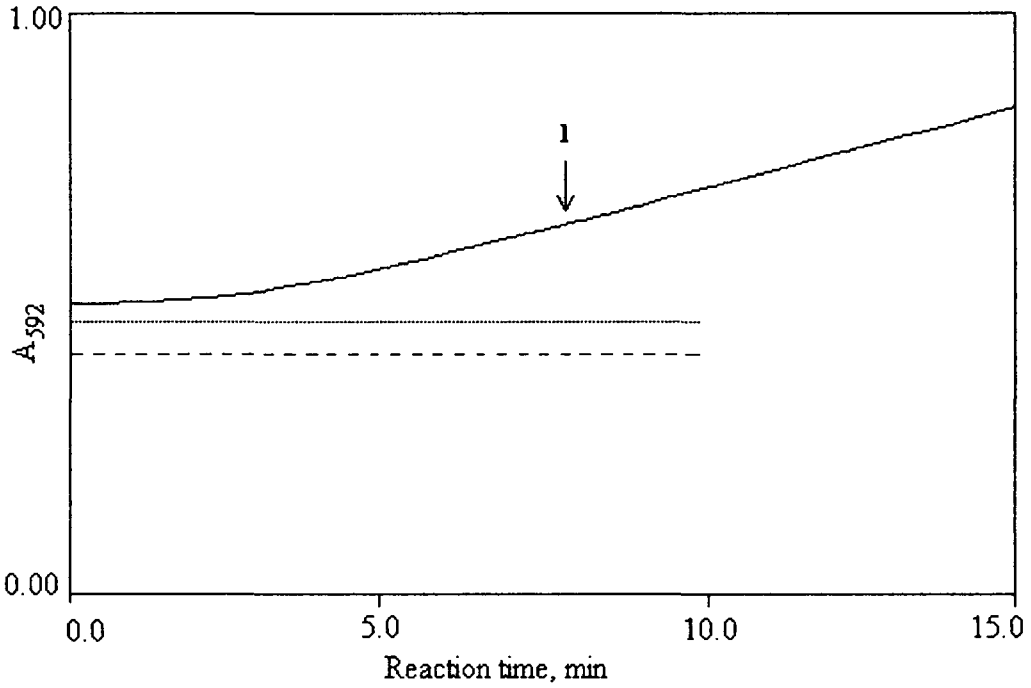

Fig. 6.5. Bromination activity with dinuclear peroxovanadate complex **5.1**. The reaction mixture contained phosphate buffer (50mM, pH 5.5), KBr (2M) and phenol red (20 μM). **1**- The increase of A_{592} indicating the secondary rate of bromination; Addition of H_2O_2 (1 mM) (·····) or EDTA (1 mM) (------) instantly stopped the secondary rate of bromination.

6.3.5 NADH is not oxidized by peroxovanadate compound 5.1 or 5.4

Under the conditions given above, a mixture of DPV and $VOSO_4$ rapidly oxidizes NADH[24]. Compound **5.1** and **5.4** were chosen as representative for testing this effect.

We now find negligible decrease in A_{340} of NADH on addition of solid compound **5.1** or **5.4** to give final concentrations of 0.37 and 0.86 mM to solutions of

NADH. There was no instant high oxidation as observed when a solution of $VOSO_4$ (0.1mM) was added to a mixture containing NADH and DPV (0.1 mM) (*Fig. 6.6*).

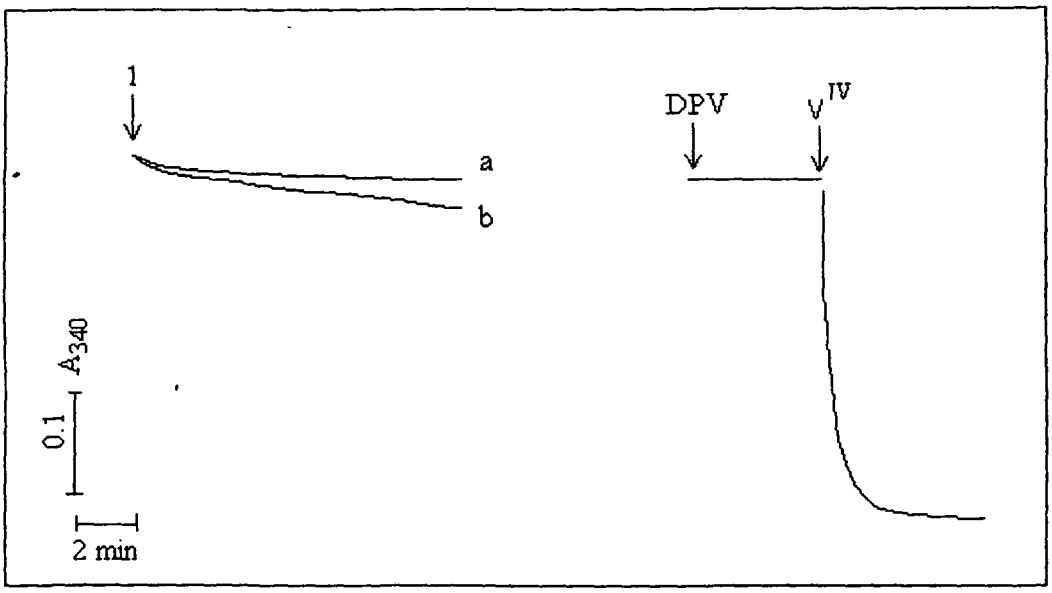

Fig 6.6. Lack of oxidation of NADH by compound **5.1**. Solid compound **5.1** was added as indicated (a) 0.37 mM; (b) 0.86 mM to a reaction mixture containing phosphate buffer (50 mM, pH 7.0) and NADH (0.2 mM) and the absorbance at 340 nm was recorded. A slow rate was found in contrast to rapid decrease obtained with a mixture of DPV (0.1 mM) and vanadyl sulphate (V^{IV}, 0.1 mM).

6.3.6 Glucose oxidase in not inactivated by peroxovanadate complex 5.1 or 5.4

Addition of DPV (0.4 mM) followed by $VOSO_4$ (0.4 mM) to a solution of glucose oxidase (0.16 mg protein/ml) inactivated the enzyme extensively[25]. Individually these reagents had no effect. The synthetic compound **5.1** or **5.4** was added as solid to a

solution of the enzyme to give final concentrations of 0.5-1.5 mM and the mixture was preincubated for 10 min. An appropriate aliquot of this mixture containing treated glucose oxidase (0.16 mg protein/ ml) to the reaction medium in the oxygraph showed nearly the same rate of oxygen consumption as the control (*Fig. 6.7*) indicating the enzyme was unaffected by the treatment.

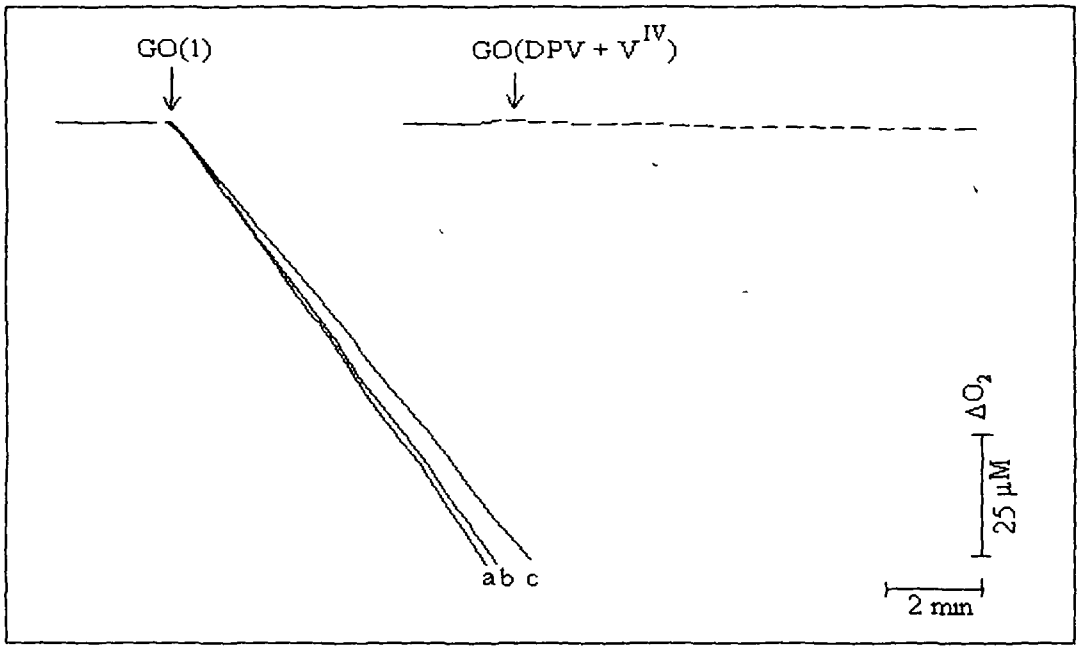

Fig. 6.7. Lack of inactivation of glucose oxidase by compound **5.1**. Solid compound **5.1** was added as indicated (a) nil; (b) 0.5 mM; (c)1.5 mM or DPV (0.4 mM) followed by vanadyl sulphate (V^{IV}, 0.4 mM)) were added to a reaction mixture containing phosphate buffer (50 mM, pH 7.0) and glucose oxidase (1.6 mg protein) and incubated for 10 min at 30°C. An aliquot containing 8 mg protein was then transferred to the oxygraph reaction vessel (1.75 ml) containing phosphate buffer (50 mM, pH 7.0) and glucose oxidase (10 mM) and oxygen consumption was followed. Extensive inactivation occurred only with DPV+V^{IV}

6.4 DISCUSSION

The redox chemistry of vanadyl, vanadate and peroxovanadates described earlier by Brooks and Sicilio[5], Jaswal and Tracey[8] and Ramasarma et al.[14,26] are pertinent in explaining the reactions involved in the present study. A scheme of reactions shown in *Fig. 6.8* is proposed which is based on our results and work of some other laboratories[5,8,10,13,14,26].

The μ-peroxo group of 'VOOV' moiety appears to be amenable for reductive cleavage by bromide that produces a bromination competent intermediate, -BrOVO(O_2), that can transfer the bromine atom to the substrate AH (reaction **a** and **b** in *Fig 6.8*). On its dissolution the compound dissociates into MPV and DPV species (*Fig 6.8* reaction **c**) as identified by ^{51}V-NMR with subsequent loss of instant bromination activity. These observations and the marginal effects of H_2O_2 and EDTA, which considerably inhibit vanadate dependent bromination activity, lend support to earlier proposal that 'VOOV" is the active group and is the likely primary oxidant of bromide at near neutral pH. The secondary bromination activity results from the formation of DPV and vanadate formed as dissociation products of the dinuclear complex in solution (*Fig 6.8*, reaction **d**). Dismutation of MPV generates vanadate which is then reduced to vanadyl by bromide (reaction **d** and **e**) as demonstrated experimentally in earlier work[14]. Vanadyl then complexes with DPV to form a μ-peroxovanadate dimer[5] (reaction **f**). This complex can then oxidize bromide and produce a bromination competent intermediate that can be recycled till phenol red is completely consumed (reaction **g**). The striking feature of the essentiality of vanadyl V(IV) for converting DPV into an effective bromide oxidant[14] was thus accommodated

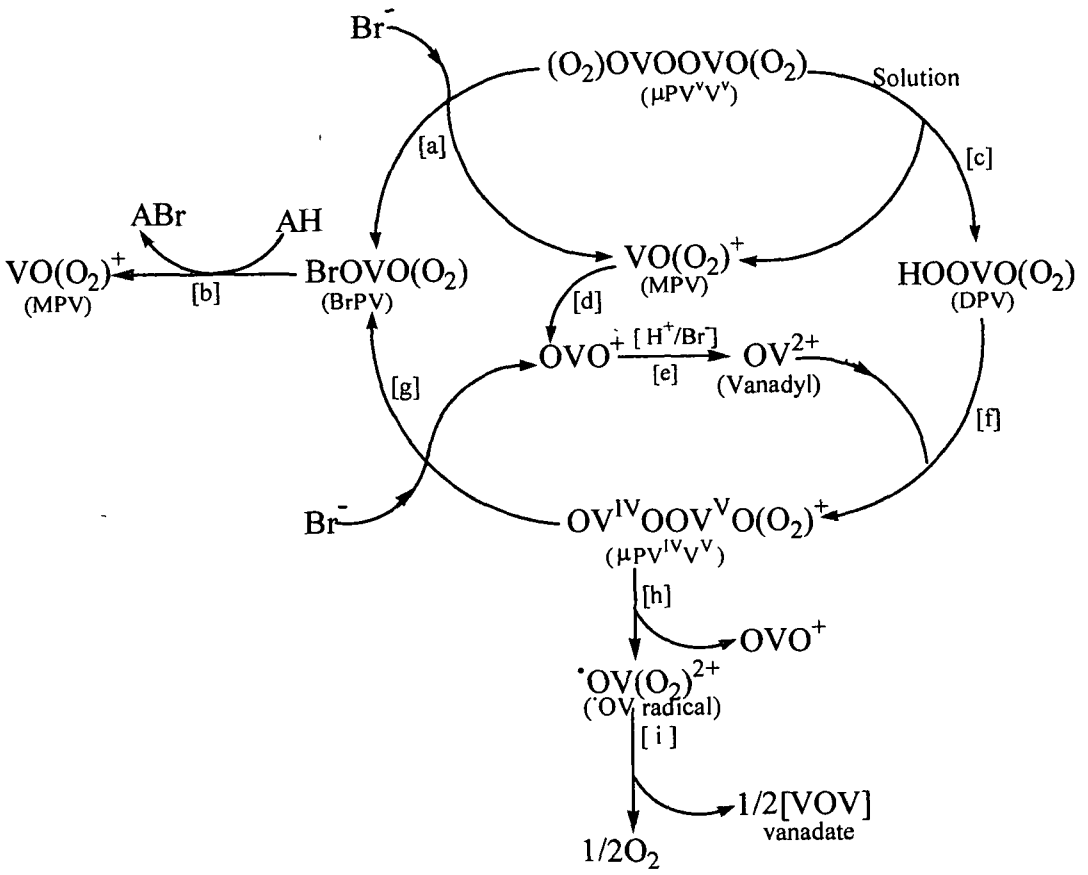

Fig. 6.8. Schematic representation of reactions occurring with peroxo-bridged divanadate compounds: (a)formation of active bromine compound BrPV and MPV when solid is added to a bromide solution; (b) transfer of bromine to acceptor AH (phenol red); (c) dissociation of the dinuclear complex on dissolution in water into MPV and DPV (in absence of bromide) with concomitant loss of bromination activity; (d) reduction of MPV to vanadate;(e) reduction of vanadate to vanadyl by acid and bromide; (f) formation of μ-peroxo compound from DPV + vanadyl; (g) breakdown of μ-peroxo group and oxidation of bromide yielding BrPV; (h) breakdown of μ-peroxo group in absence of bromide; (i) dismutation of •OV radical releasing O_2. Peptide ligands in the compounds are not shown. Valancy state of reduced vanadium is shown as $_V{}^{IV}$ and all others are V^V. No attempt is made to show the exact stoichiometry of the reaction.

as V^{IV}-O-O-V^{V} in the reaction pathway in *Fig 6.8*. Bromide is needed in two steps, in recycling vanadyl by reduction of vanadate and in interacting with peroxo-bridged intermediate, which explains the slow reaction being second order with respect to KBr concentration. Inhibition of secondary reaction in presence of excess H_2O_2 is evidently owing to the conversion of vanadate into DPV which is inactive in bromination. EDTA inhibits reaction **g** by complexing with V(V) and V(IV) centers of the μ-peroxovanadate intermediate as was demonstrated experimentally in earlier studies.

Reaction **c** shows the breakdown of compounds **5.1-5.4** when solids were added to water in absence of bromide. The reaction sequence **c-f-h-i**, shown in *Fig 6.8*, indicates the way dioxygen is released when solid compound was added to water. The process involves interaction of DPV with vanadyl (reaction **f**), formed from vanadate (reaction **e**) in acid medium and decomposition of the μ-peroxo complex (reaction **h** and **i**). Formation and decay of (reaction **h** and **i**) of oxygen radical species of peroxovanadate are implicated in this process as described in earlier reports[5,24,31]

The present experiments confirm the reactivity of the synthetic peroxo-bridged compounds in producing bromination competent intermediate. The compounds were unable to oxidize NADH or inactivate glucose oxidase, the two activities shown by a mixture of diperoxovanadate and vanadyl. This demonstration of peroxo-bridged divanadate as a powerful, selective oxidant of bromide, active at physiological pH, should make it a possible candidate of mimic in the action of vanadium in bromoperoxidase proteins. Since aqueous solution of the compounds did not show the instant bromination activity, in spite of the fact that *c.*50% of the peroxo groups were

retained in the products, it is evident that activities of the original compounds were limited to a transient period and lasted until breaking up of the peroxo bridged species. We are however, restrained to suggest any involvement of the afore mentioned reactions in the action of the enzymes, in absence of direct evidence. But from reports available, there appears to be a definite potential of the peroxovanadate intermediates (*Fig. 6.8*) in oxidative modifications in biological systems.

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CHAPTER 7

Synthesis of Newer Dinuclear and Mononuclear Peroxo-vanadium(V) Complexes Containing Amino Acids as Co-ligands. A Comparative Study of Some of Their Properties

Synthesis of Newer Dinuclear and Mononuclear Peroxo-vanadium(V) Complexes Containing Amino Acids as Co-ligands. A Comparative Study of Some of Their Properties *

7.1 INTRODUCTION

It is evident from results of investigations presented in Chapter 5 and 6 of this thesis, that the newly synthesized peroxo-bridged divanadate compounds of the type $[V_2O_2(O_2)_3(dipeptide)_3].H_2O$ and $[V_2O_2(O_2)_3(tripeptide)_2].H_2O$ distinguish themselves in terms of their spectral as well as solution properties from the majority of peroxovanadium complexes possessing peroxo groups bonded exclusively in a side-on fashion. Each of these complexes exhibited unique redox properties and could act as bromide oxidant with good activity at physiological pH, an essential requirement of biomimetic model.

Taking the above observations into account and in continuation of our work on dimeric peroxovanadates it was considered imperative to investigate the possible similarities or differences in spectral, redox or solution properties which may exist between the μ-peroxovanadate and monomeric peroxovanadate complexes in analogous co-ligand environment. There appears to be no literature comparing these systems. It was felt that the informations derived from such studies could provide valuable insight into the special features that may be associated with a 'VOOV' group which make complexes with such moiety more reactive than monomeric compounds with side-on

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bound peroxides.

Accordingly, we set out to synthesize additional members of dinuclear μ-peroxovanadate complexes stabilized by co-ordinatively versatile amino acids with polar side chains viz., asparagine and glutamine as heteroligands. In order to ensure that the corresponding monomeric compounds were available for comparison we have also directed our efforts to synthesize mononuclear peroxovanadates with a co-ligand environment made up of glutamine or asparagine.

The fact that the μ-peroxovanadate compounds with 'VOOV' moiety were highly active in peroxidative bromination[1] in aqueous medium at physiological pH, opened up possibilities of such complexes being useful as mild biomimetic brominating agents[2,3] when used in conjunction with inorganic bromides for organic bromination. The observations prompted us to explore the catalytic potential of these complexes in organic bromination reaction[2-8].

In this Chapter, the synthesis and physicochemical characterization of complexes of the type $[V_2O_2(O_2)_3L_3]\,H_2O$ and $Na[VO(O_2)_2L].H_2O$, (L= asparagine and glutamine) are presented. The two types of complexes synthesized provided a means of undertaking a comparative study of their various properties. Results pertaining to the activity of the complexes in mediating bromination of some aromatic substrates are also embodied herein.

7.2 EXPERIMENTAL SECTION

The chemicals used were all reagent grade products (S.D fine, E. Merck, (India), CDH and SRL). Catalase was obtained from Sigma-Aldrich Chemicals

Company Pvt. Ltd. The water used for solution preparation was deionised and distilled.

7.2.1 Synthesis of dimeric peroxovanadate complexes, $[V_2O_2(O_2)_3(asn)_3].H_2O$ (7.1) and $[V_2O_2(O_2)_3(gln)_3].H_2O$ (7.2)

In a typical reaction solid V_2O_5 (0.25g, 1.37 mM) was mixed with amino acid in a 250 ml beaker maintaining molar ratio of V: amino acid as 1:2 to which 15 ml of 30% H_2O_2 (132.3 mM) was added gradually with constant stirring. The reaction mixture was then stirred for *c.*15 minutes in an ice-bath keeping the temperature below 4°C. During this period the solid dissolved yielding a clear reddish yellow solution. The pH of the solution was recorded to be 2.0. No attempt was made to adjust the pH of the reaction solution. On adding pre-cooled ethanol (*c.* 50 ml) to the above solution under continuous stirring, an orange yellow colored pasty mass separated out. After allowing to stand for about 10 minutes in the ice-bath, the supernatant liquid was decanted, and the residue was treated repeatedly with acetone:ethanol (3:1 v/v) mixture under scratching until it became micro-crystalline solid. The product was separated by centrifugation, washed with cold ethanol and dried *in vacuo* over concentrated sulfuric acid.

7.2.2 Synthesis of monomeric peroxovanadate complexes, $Na[VO(O_2)_2(asn)].H_2O$ (7.3) and $Na[VO(O_2)_2(gln)].H_2O$ (7.4)

The procedure adopted for synthesis is common to both the complexes. This consisted of gradual addition of 12 ml H_2O_2 (30% solution, 105.84 mM) to a mixture of V_2O_5 (0.25g, 1.37 mM) and amino acid at a molar ratio of V: amino acid at 1:1 with

continuous stirring. Keeping the temperature below 4°C in an ice-bath, the mixture was stirred for *c.*15 minutes until all solids dissolved. At this stage the pH of solution was recorded to be *c.*2. Concentrated sodium hydroxide solution (conc. *c.*8M) was added dropwise with constant stirring to raise the pH of the reaction medium to *c.*5. On adding pre-cooled ethanol (about 50 ml) to this mixture under vigorous stirring a yellow colored pasty mass separated out. After standing for about 15 minutes in the ice-bath, the supernatant liquid was decanted, and the residue was treated repeatedly with acetone:ethanol (3:1 v/v) mixture under scratching until it became microcrystalline solid. The product was separated by centrifugation, washed with cold ethanol and dried *in vacuo* over concentrated sulfuric acid.

7.2.3 Elemental analysis

The compounds were analyzed for vanadium, peroxide, sodium, carbon, hydrogen and nitrogen by the methods mentioned in Chapter 2. The analytical data are presented in Table 7.1.

7.2.4 Spectroscopic measurements

Spectroscopic measurements were performed by using instruments and methods described in Chapter 2. Structurally significant IR and UV bands and their assignments are reported in Tables 7.2, 7.3 and 7.5. ^{1}H-NMR spectra of the organic reaction products were recorded in deuterated chloroform. Chemical shift values of the products are presented in Table 7.7.

Table 7.1. *Analytical data of synthesized peroxovanadate complexes 7.1-7.4*

No.	Compound		(% calculated) % found					Approximate yield(%)
		Na	C	N	H	V	O_2^{2-}	
7.1	$[V_2O_2(O_2)_3(asn)_3].H_2O$	-	(22.36)	(13.00)	(3.83)	(15.82)	(14.91)	
		-	22.59	13.10	4.01	15.82	14.90	50
7.2	$[V_2O_2(O_2)_3(gln)_3].H_2O$	-	(26.24)	(12.24)	(4.37)	(14.87)	(13.99)	
		-	26.10	12.30	4.50	14.80	14.10	47
7.3	$Na[VO(O_2)_2(asn)].H_2O$	(7.56)	(15.79)	(9.21)	(2.30)	(16.77)	(21.05)	
		7.69	15.44	9.15	2.23	16.89	20.90	43
7.4	$Na[VO(O_2)_2(gln)].H_2O$	(7.23)	(15.78)	(9.21)	(2.63)	(16.03)	(20.12)	
		7.32	15.62	9.29	2.49	16.34	20.04	40

7.2.5 Stability of complexes in solution - measurement of oxygen release from the peroxo-vanadium complexes

A Gilson 5/6 H oxygraph fitted with a Clark oxygen electrode was used for measuring changes in the concentration of dissolved oxygen (0.224 mM at 30^0C) in the medium by the method given in Chapters 2 and 6. Results of oxygen release reactions are presented in Table 7.4.

7.2. 6 Measurement of bromination activity in aqueous solution

Bromination activity of the synthesized peroxo-vanadium compounds in aqueous solution was studied by the method mentioned Chapter 2. The data on bromination activity of the compounds are given in Table 7.6.

7.2.7 Bromination of organic substrates and products analysis

In a representative procedure, organic substrate (0.5 mM) was added to a solution of acetonotrile : water (1:1) (3 ml) containing KBr (1.5 mM). Weighed amount of solid peroxovanadate complex **7.1** or **7.2** (0.25 mM) was then added to the reaction mixture at room temperature under continuous stirring. The stirring was continued for *c.*1 hr. 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. Spectroscopic studies and melting point determinations were made to interpret the products.

7.3 RESULTS AND INTERPRETATION

7.3.1 Synthesis and characterization

Orange coloured dinuclear peroxovanadate complexes **7.1** and **7.2** were obtained by adopting a synthetic protocol similar to the one established for the synthesis of peptide containing complexes **5.1-5.4**, outlined in Chapter 5. The synthetic methodology, which afforded the dimeric complexes with $V_2O_2(O_2)_3$ moiety stabilized by amino acids occurring as zwitterions, was based on the reaction of V_2O_5 with H_2O_2 and the respective amino acid in aqueous solution of pH *c*.2. A comparatively higher pH of *c*.5 of the reaction medium is likely to be responsible for the formation of yellow alkali oxodiperoxovanadate complexes **7.3** and **7.4**, as it is known that in a solution of vanadate and excess H_2O_2 at pH>5 formation of diperoxoxvanadate (DPV) species is favoured[9-11].

In solid state complexes **7.1** and **7.2** were found to be stable for a few weeks whereas compounds **7.3** and **7.4** remained stable for months stored dry at <20°C. But they tended to be hygroscopic at ambient conditions and decompose in a few days. The compounds **7.1-7.4** were diamagnetic in nature as evident from the magnetic susceptibility measurements, in conformity with the presence of vanadium(V) in each of them.

From the elemental analysis data, the ratio of $V:O_2^{2-}$ and V : amino acid was ascertained to be 2:3 for complexes **7.1** and **7.2** in clear agreement with their formulation as $[V_2O_2(O_2)_3L_3].H_2O$. The elemental analysis results for the complexes **7.3** and **7.4** indicated the presence of two peroxide groups and one amino acid per vanadium centre which could be fitted with the formula $Na[VO(O_2)_2L].H_2O$.

Each of the amino acid containing triperoxodivanadates(V) and mononuclear diperoxovanadate complexes displayed characteristic spectral pattern in the Infrared region (*Fig. 7.1* and *Fig.7.2*) involving absorptions due to ν(V=O), co-ordinated peroxide, amino acid ligands and lattice water. Presence of terminally bonded V=O group[10,12] was ascertained from the characteristic strong absorption consistently appearing at *c.*960 cm^{-1} in the spectra of each of the complexes.

The peroxo bands observed in the spectra of the complexes **7.3** and **7.4** (Table 7.2 and 7.3), were in the range characteristic of triangularly bonded peroxide[12]. In the spectra of the complexes **7.1** and **7.2**, appearance of two ν(O-O) bands at *c.*870 cm^{-1} and at *c.*810 cm^{-1} causes us to believe that the complexes, like the ones reported in Chapter 5, have structurally two different types, viz., side-on bound and the bridging bidentate, of peroxo groups[13-15]. Significantly, no band appeared in the *c.*810 cm^{-1} region in the spectra of the monomeric complexes **7.3** and **7.4** (*Fig. 7.1* and *Fig.7.2*). These results thus support the original formulations of the compounds **7.1-7.4**.

Existence of co-ordinated amino acids in the complexes **7.1-7.4** was evident from their IR spectra which showed characteristic differences between the spectral pattern originating from amino acids of the complexes and the spectra of the free amino acids[16,17]. The N-H stretching bands were observed in the 3300-3000 cm^{-1} region as expected from the $-N^{+}H_3$ group and the amino group of the amino acid side chains. The rocking modes of $N^{+}H_3$ occurred at *c.*1150 and *c.*1060 cm^{-1}. A band appearing at 1670-1660 cm^{-1} has been assigned to ν(C=O) mode of the amide group of the amino acid side chain. Participation of the band in hydrogen bonding appeared possible from its slight shift with respect to free ligand value accompanied by broadeing. Absorption

attributable to ν_{as}(COO) of the co-ordinated carboxylate group appeared in the 1650-1640 cm^{-1} region in the spectra of the complexes[16]. Symmetric stretching vibration of carboxylate group of uncoordinated asparagine and glutamine occur at 1430 and at 1411 cm^{-1}, respectively. The spectra of complexes **7.1** and **7.2** displayed a medium intensity band at *c.*1360 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 co-ordination of carboxylate group via O(carboxylate) atom[16]. The spectra of the complexes **7.1** and **7.2** exhibited another distinct band at 1412 cm^{-1} (complex **7.1**) and at 1405 cm^{-1} (complex **7.2**) attributable to a bridging carboxylate group (ν_{as}-$\nu_s \approx 200$ cm^{-1}). Symmetric stretching frequency of co-ordinated carboxylate group of the amino acid ligands in complexes **7.3** and **7.4,** on the other hand, was found to be shifted to a higher value of 1435 and 1420 cm^{-1}, respectively compared to their free ligand value. The spectra of these complexes displayed the ν_{as}(COO) in the 1640-1635 cm^{-1} region. The shift of ν_s(COO) band to a higher frequency and the decrease in Δ value [$\Delta \approx \nu_{as}$(COO) - ν_s(COO)] is typical of a carboxylate group bonded in a bidentate fashion[16]. The presence of asparagine and glutamine as zwitterion in these complexes with protonated -N^+H_3 was ascertained from the spectrum as mentioned in the foregoing discussions. Presence of lattice water in each of the complexes, **7.1-7.4** 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 each of the complexes **7.1-7.4**, amino acid occurring as zwitterion binds the V(V) centers through O(carboxylate) atoms.

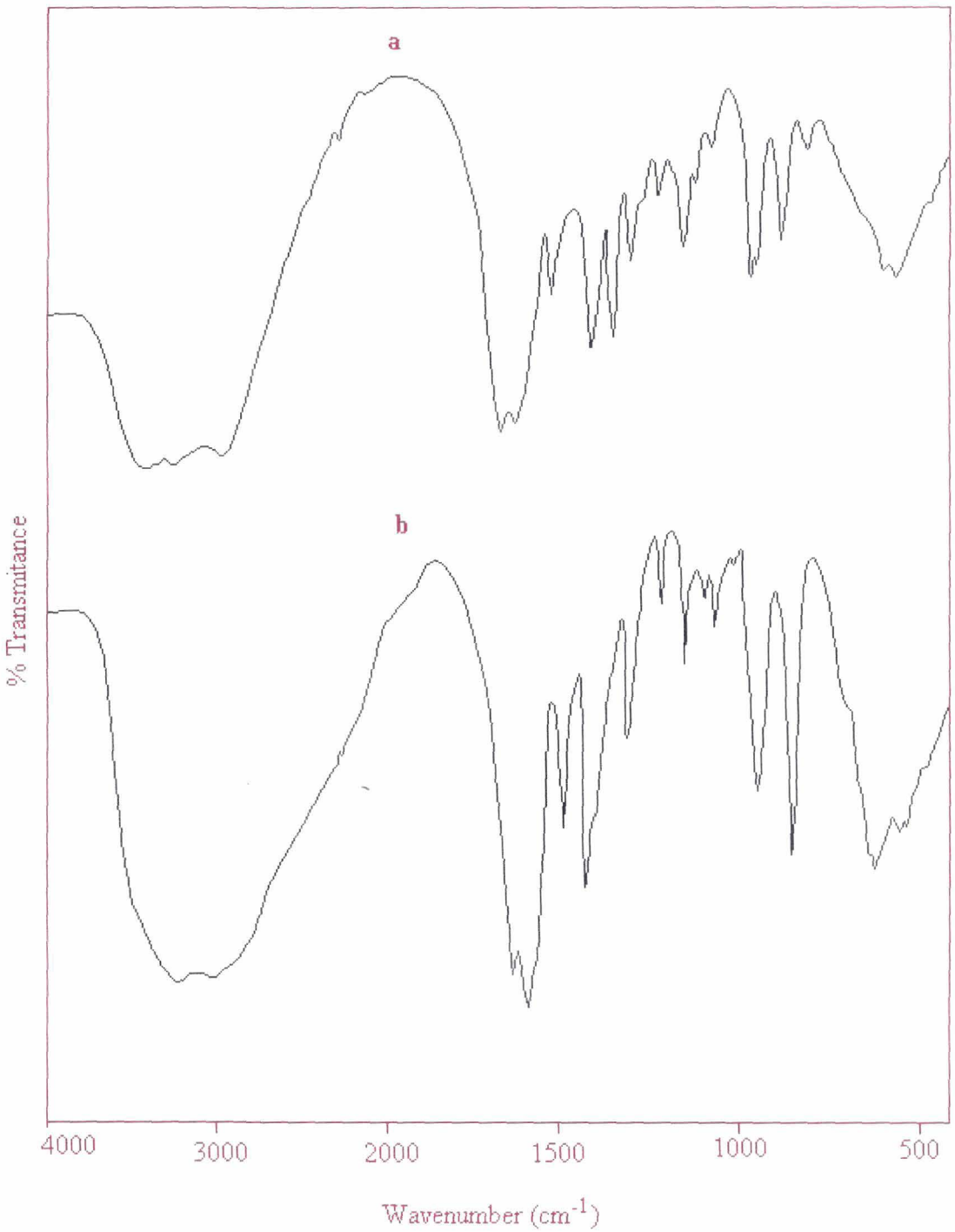


Fig. 7.1 IR spectra of (**a**) $[V_2O_2(O_2)_3(asn)_3].H_2O$ and (**b**) $Na[VO(O_2)_2(asn)].H_2O$.

Table 7.2 *Structurally significant IR bands of* $[V_2O_2(O_2)_3(asn)_3].H_2O$ *and* $Na[VO(O_2)_2(asn)]\ H_2O$

IR bands (cm^{-1})		Assignment
$[V_2O_2(O_2)_3(asn)_3]\ H_2O$ (**7.1**)	$Na[VO(O_2)_2(asn)].H_2O$ (**7.3**)	
3500-3400	3500-3400	ν(O-H)
3300-3000	3300-3100	ν(N-H)
1669	1670	ν(C=O)
1650-1640	1640-1635	ν_{as}(COO)
1412	1435	ν_s(COO)
1355	-	
1151	1150	$\rho_r(N^+H_3)$
1079	1074	
954s	949s	ν(V=O)
859s	869s	ν(O-O)
803 w	-	
631	640	$\nu_{as}(V\text{-}O_2)$
562	557	$\nu_s(V\text{-}O_2)$

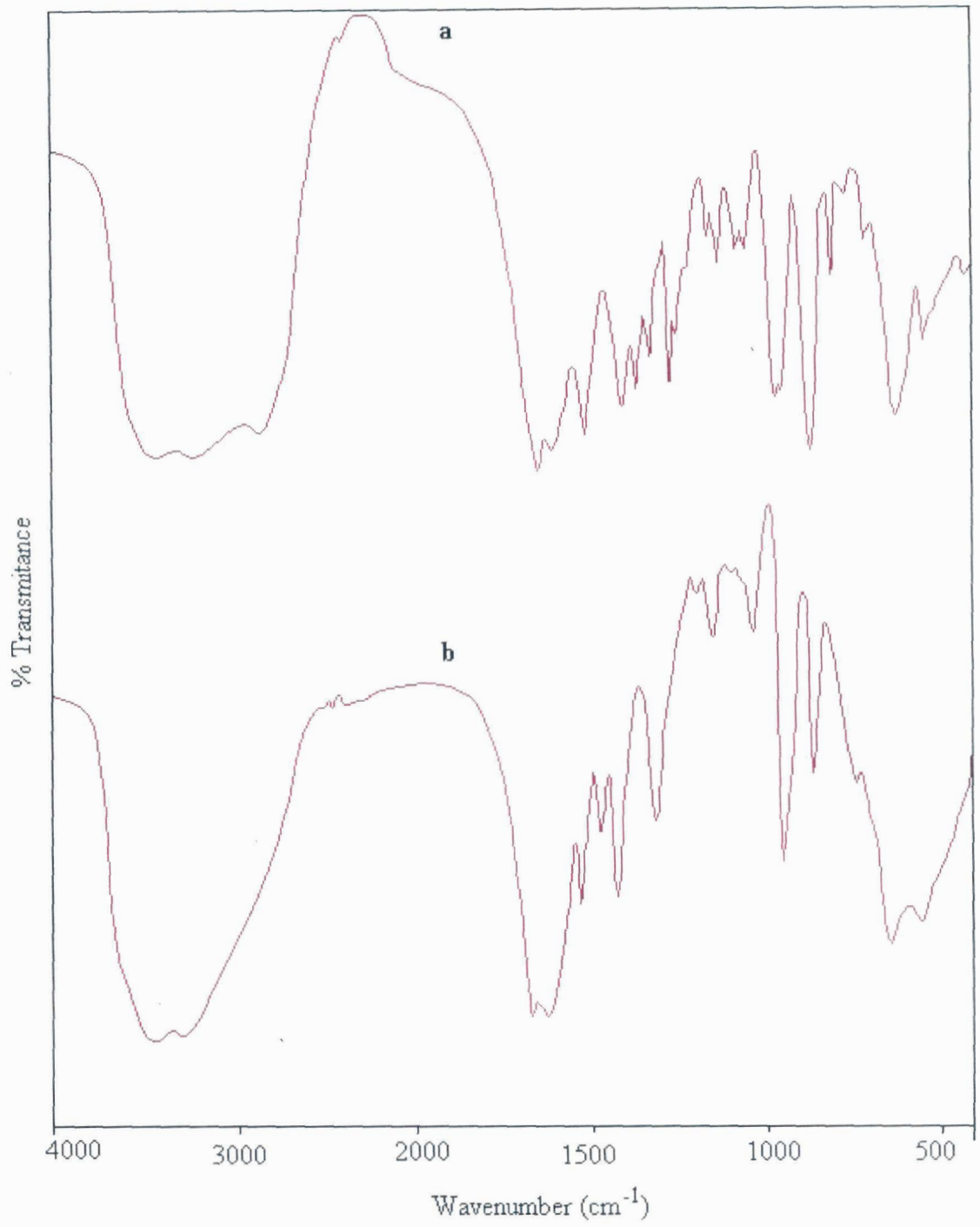


Fig. 7.2 IR spectra of (**a**) $[V_2O_2(O_2)_3(gln)_3].H_2O$ and (**b**) $Na[VO(O_2)_2(gln)].H_2O$.

Table 7.3 *Structurally significant IR bands of* $[V_2O_2(O_2)_3(gln)_3].H_2O$ *and* $Na[VO(O_2)_2(gln)].H_2O$

IR bands (cm^{-1})		Assignment
$[V_2O_2(O_2)_3(gln)_3]\,H_2O$ (**7.2**)	$Na[VO(O_2)_2(gln)].H_2O$ (**7.4**)	
3500-3200	3500-3200	ν(O-H)
3300-3000	3300-3100	ν(N-H)
1670	1670	ν(C=O)
1650-1640	1640-1635	ν_{as}(COO)
1405	1420	ν_s(COO)
1359	-	
1151	1163	$\rho_r(N^+H_3)$
1059	1055	
955s	953s	ν(V=O)
870s	872s	ν(O-O)
809	-	
640	660	$\nu_{as}(V\text{-}O_2)$
562	540	$\nu_s(V\text{-}O_2)$

Based on these observations a structure of the type shown in *Fig.7.3(a)* has been proposed for complexes **7.1** and **7.2**, which is shown with asparagine complex as a representative. For complexes **7.3** and **7.4** the structure of the type shown in *Fig.7.3(b)* has been envisaged.

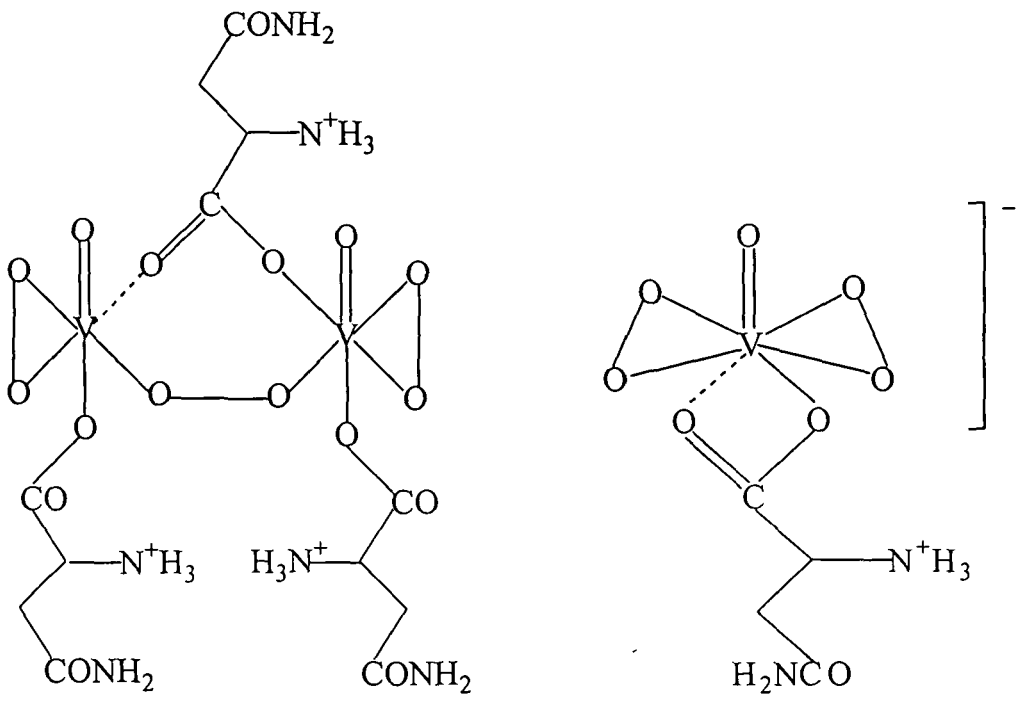


Fig. 7.3 Proposed structures of peroxovanadate-amino acid complexes. (**a**) structure of dimeric peroxovanadate compounds shown with $[V_2O_2(O_2)_3(asn)_3].H_2O$ as representative, (**b**) structure of monomeric peroxovanadate compounds shown with $Na[VO(O_2)_2(asn)].H_2O$ as representative.

7.3.2 Nature and stability of the complexes in solution

Investigations involving measurement of oxygen released from the complexes, determination of peroxide content as function of time, their ^{51}V-NMR as well as electronic spectroscopic measurements revealed that the monomeric diperoxovanadate complexes, **7.3** and **7.4** possess much higher stability in solution as compared to the dinuclear complexes, **7.1** and **7.2**. Complexes **7.1** and **7.2**, like their peptide containing analogues, were rather unstable in water and degradative loss of the peroxide groups was evident from the oxygen evolution taking place when the compounds were added to water. Immediately on adding the complexes (*c.*2 mg/ml) the rate of oxygen release was observed to be exceedingly high of about 10μM /min for a brief period of *c.*15 min.

Close resemblance between compounds **7.1** and **7.2** and peptide containing peroxovanadates **5.1-5.4**, in terms of their peroxide content and nature in solution were further apparent from their activity with catalase. Slow release of oxygen on addition of catalse, indicated that the products in such solutions were peroxovanadates (Table 7.4). The subsequent secondary rates of oxygen release, calculated from the data, paralleled the residual peroxide concentrations. Due to the initial loss of peroxide during the process of solution preparation lower values of 0.7 and 0.4 O_2 per mole of the compound were recorded.

The extent and rates of oxygen released under the effect of catalase action from the complexes **7.3** and **7.4** (Table 7.4) were found to be comparable to that of aquo oxodiperoxvanadate complex[18] (DPV) indicating their similarity with respect to number of peroxide and probably the pattern of their co-ordination to the V(V) centre. On

incubation with catalase DPV was found to be degraded releasing half the molecular equivalent of oxygen[18] at a rate of 36 μM/min from a solution of 0.2 mM.

The stability of the complexes **7.1-7.4** were also studied by estimating the peroxide content of the standard solution of the compounds at different time intervals which revealed the variation in stability of the complexes. Whereas compounds **7.1** and **7.2** lost its peroxide rapidly, compounds **7.3** and **7.4**, in contrast, remained stable for a period of 24 hr or more.

The electronic spectra of the compounds **7.1-7.4** in aqueous solution exhibited a weak intensity broad LMCT band at 310-320 nm (Table 7.5) originating from co-ordinated peroxide. In case of the complexes **7.1** and **7.2** the intensity of this band was found to decrease with time consistent with the loss of peroxide from these complexes and their unstable nature.

Table 7.4 *Catalase dependent oxygen release from peroxovanadium compounds*

No.	Compound	Conc. mM	Oxygen release		ΔO_2/compd.
			μM/min	Total, μM	(mol. ratio)
7.1	$[V_2O_2(O_2)_3(asn)_3].H_2O$	0.2	17.6	145	0.72
7.2	$[V_2O_2(O_2)_3(gln)_3]\,H_2O$	0.2	11.2	87	0.44
7.3	$Na[VO(O_2)_2(asn)].H_2O$	0.2	32.2	185	0.92
	DPV	0.2	36.0	192	0.96

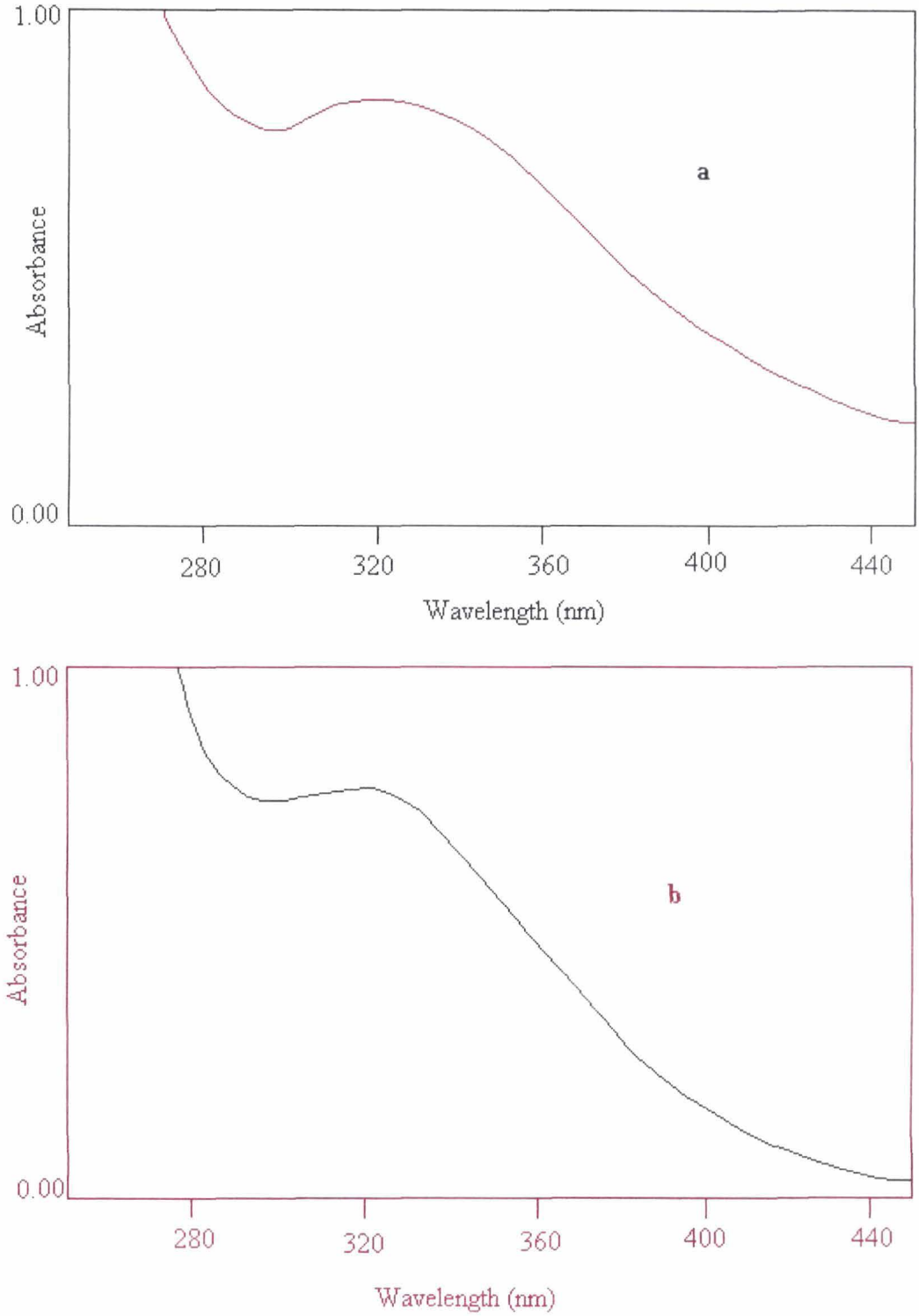


Fig. 7.4 UV spectra of (**a**) $[V_2O_2(O_2)_3(asn)_3].H_2O$ and (**b**) $Na[VO(O_2)_2(asn)].H_2O$.

Table 7.5 *Ultraviolet spectral data of the peroxovanadate complexes 7.1-7.4*

No.	Compound	UV peak	
		nm	A(1mM)
7.1	$[V_2O_2(O_2)_3(asn)_3]\,H_2O$	320	0.70
7.2	$[V_2O_2(O_2)_3(gln)_3]\,H_2O$	328	0.40
7.3	$Na[VO(O_2)_2(asn)].H_2O$	324	0.63
7.4	$Na[VO(O_2)_2(gln)].H_2O$	321	0.72

7.3.3 ^{51}V-NMR spectral analysis of aqueous solution of compounds **7.1-7.4**

Using ^{51}V-NMR spectroscopy and assigning the peaks observed based on the data available[19-22], major species present in the solutions of compounds **7.1-7.4** were identified. From the NMR spectral studies it was further evident that peroxo-bridged dimeric complexes **7.1** and **7.2** undergo rapid degradation in water. A close similarity was observed between the NMR spectral pattern of dinuclear compounds **7.1** and **7.2** and of peptide containing compounds **5.1-5.4** causing us to infer that the two sets of compounds behave similarly in solution. The three peaks at -427, -509 and -527 ppm with intensity ratio of 1:2:2, correspond to V_{10}. The major peak at -694 ppm, and the

less intense peaks at -545 ppm and -650 ppm were identified with diperoxovanadate and monoperoxovanadate species, respectively. Free vanadate (V_1), formed on depletion of its peroxide, is known to oligomerize to decameric form (V_{10}) in solution. Slight variations, in some cases, of the observed chemical shifts from those reported in the literature are probably caused by variations of pH and presence of co-ordinated ligands in some of the products. The two weak resonances appearing near -714 ppm may be assigned to diperoxovanadate species containing amino acid ligand[22,23].

In contrast to the observance of several peaks in the spectra of compounds **7.1** and **7.2,** the spectra of complexes **7.3** and **7.4** displayed a predominant peak at -695 ppm characteristic of a diperoxovanadate species. Indication of presence of traces of vanadates appeared in the spectra of the mononuclear complexes recorded after 30 min of preparation of solutions.

7.3.4 Redox activity of the complexes **7.1-7.4** in bromination reaction

The bromination activity of the compounds **7.1-7.4** was tested by adding weighed amount of the solid to the reaction medium of bromide in phosphate buffer with phenol red as substrate, following the method outlined in Chapters 2 and 6. Peroxo-bridged dimeric compounds **7.1** and **7.2**, like the other μ-peroxovanadate complexes described in Chapter 5, were found to be highly active in mediating bromination of the substrate phenol red. Referred as 'instant' activity, this can be visualized by the solution turning blue immediately after adding the solid compounds **7.1** or **7.2** and quantitated by the jump in A_{592} (*Fig 7.5*). After the initial burst of bromination, these reaction mixtures contained DPV and some free vanadate. As

expected the two compounds together gave a secondary rate of bromination seen as progressive increase in A_{592} indicating increase in the amount of the product (*Fig 7.6*). The data on bromination activities of the compounds **7.1** and **7.2**, which compare very well with those derived from similar studies on compounds **5.1**-**5.4** (vide Chapter 5), are presented in Table 7.6.

Significantly, the monomeric diperoxovanadate compounds **7.3** and **7.4** were totally inactive in bromination under identical conditions. Under these conditions DPV (1mM) was inactive on its own, however, in presence of vanadate showed the secondary rate but not the instant activity.

The information derived from the above studies support the proposal that an active group present in the peroxo-bridged complexes **7.1** and **7.2** is responsible for the ready oxidation of bromide and subsequent bromination of the substrate. In spite of their being highly unstable in solution, the instant activity of 37-43% recorded confirms the oxidising ability of the original compounds.

7.3.5 Substrate bromination in aqueous-organic media – evidence for electrophilic bromination

In order to examine the catalytic potential of the complexes in organic bromination, complex **7.1** or **7.2** was added to a solution of acetonotrile : water (1:1) containing substrate and inorganic bromide (KBr) and stirred for *c.*1 hr at room temperature. The reactions were carried out in absence of buffer. Several activated aromatics were transformed into their corresponding bromo-organics in presence of peroxo-bridged divanadate complexes **7.1** or **7.2** (Table 7.7). Preferential bromination at

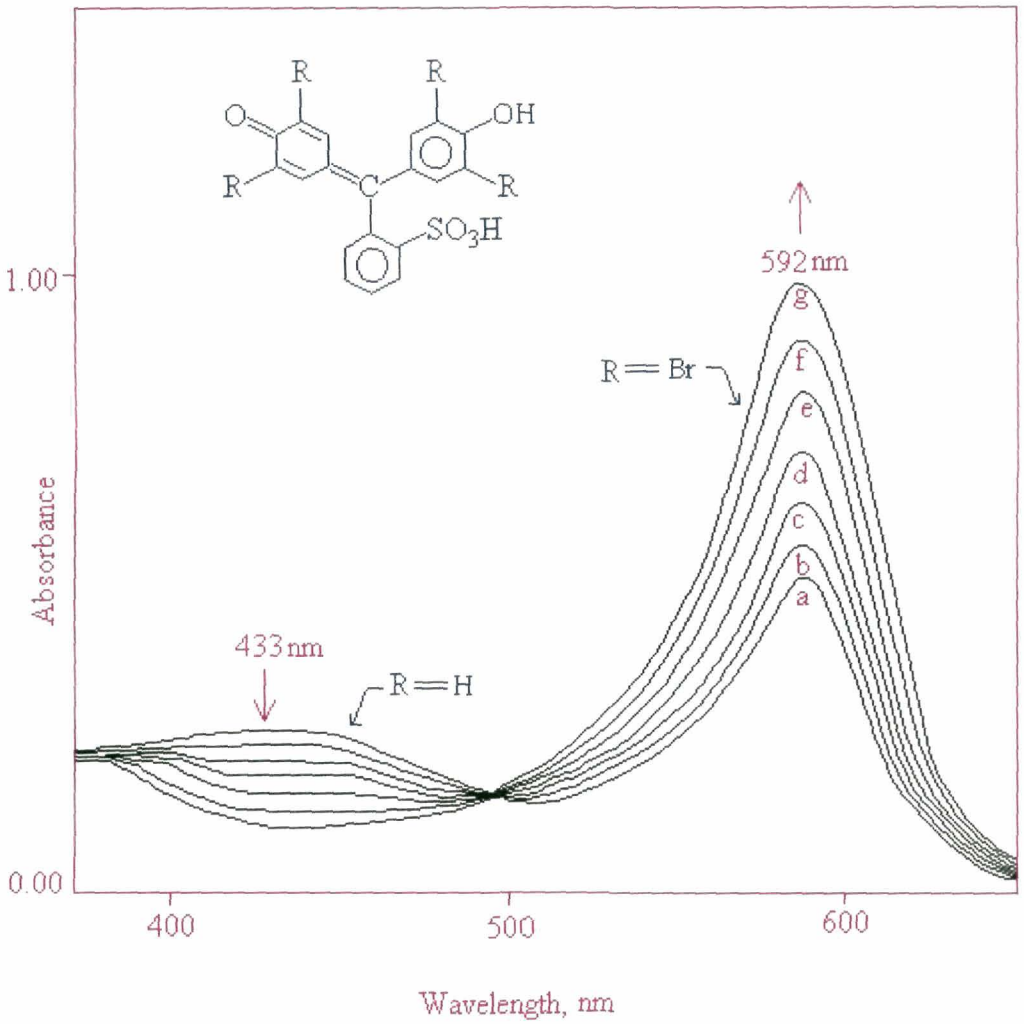


Fig. 7.5 Bromination activity with dinuclear peroxovanadate complex **7.2**. a. The spectrum recorded immediately after adding the solid compound to the reaction mixture showing the "instant activity"; b-g. Spectral changes at 5 minutes interval. The reaction mixture contained phosphate buffer (50 mM, pH 5.5), KBr (2M) and phenol red (20 μM).

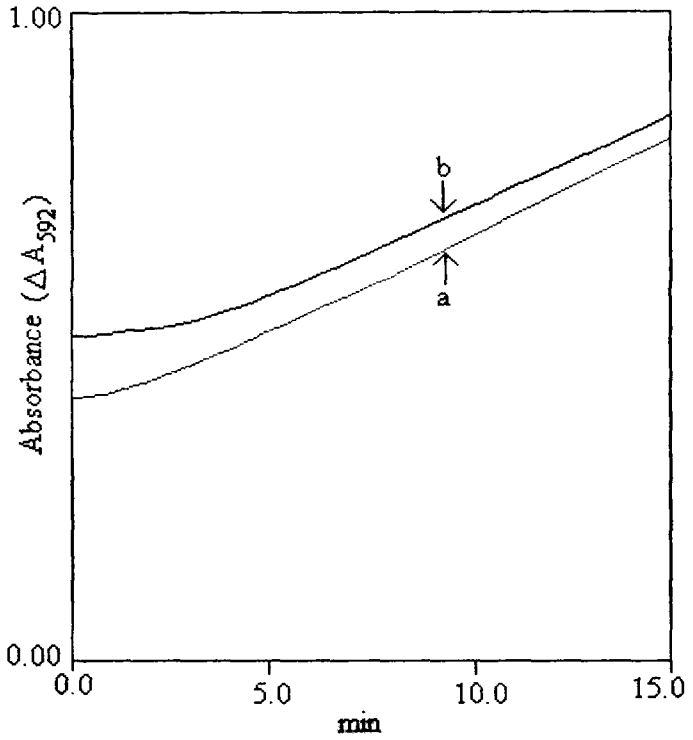


Fig.7.6 The increase of A_{592} indicating the secondary rate of bromination: a. with complex **7.1**; b. with complex **7.2**. The reaction mixture contained phosphate buffer (50 mM, pH 5.5), KBr (2M) and phenol red (20 μM).

Table 7.6 *Bromination of phenol red with peroxovanadate complexes 7.1 and 7.2*

No.	Compound	Conc. mg/ml	mM	Instant activity ΔA_{592}	bromine transfer total, μM	bromine transfer μM/mM compd.	Secondary rate (extrapolated to 1mM compound) μM Br/min
7.1	$[V_2O_2(O_2)_3(asn)_3].H_2O$	0.10	0.15	0.406	24.09	160.6	7.05
7.2	$[V_2O_2(O_2)_3(gln)_3].H_2O$	0.12	0.17	0.535	31.74	86.7	7.29

either ortho or para position of the aromatic ring leading to mono substitution indicate an electrophilic bromination mechanism.

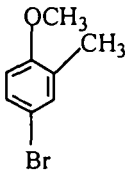
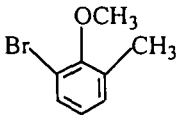
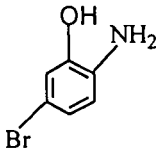
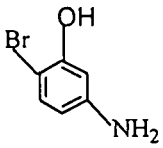
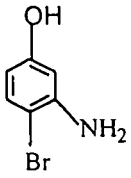
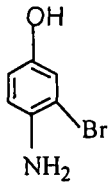
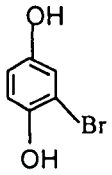
Further mechanistic information about the reaction came from the study with the substrate 2-methoxytoluene, specially chosen for the purpose. That the brominating species was 'Br^+' and not a Br· radical in these reactions was evident from the exclusive formation of ring substituted products, 3- or 5-bromo-2-methoxy toluene. Bromination through radical reaction would have produced benzyl bromide[24]. The identity of all products was confirmed by melting point determination, comparison of their ^{1}H-NMR spectra (Table 7.7) with authentic samples and also by HPLC analysis. Attempted bromination using complexes **7.3** or **7.4** under similar reaction conditions did not yield brominated products as anticipated.

7.4. DISCUSSION

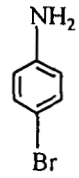
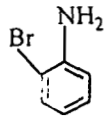
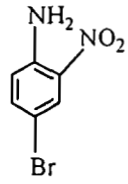
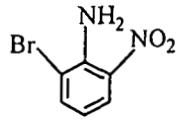
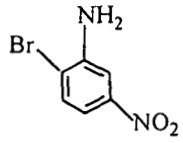
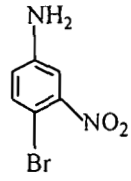
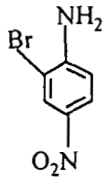
The reaction of vanadium with H_2O_2 is highly sensitive to pH and it is known that the number of peroxo groups per vanadium center increases with increasing pH of the reaction solution[9,25,26]. In the present study, it has been possible to isolate two types of peroxovanadates viz., molecular dimeric complexes $[V_2O_2(O_2)_3L_3]H_2O$ and anionic monomeric complexes $Na[VO(O_2)_2L].H_2O$, (L= asparagine or glutamine), by varying the pH of the reaction medium.

One of the notable common features between divanadate compounds **7.1**, **7.2** and **5.1-5.4** is the presence of three heteroligands per two vanadium atoms in the co-ordination sphere of the complexes. In each of these compounds two of the ligands

Table 7.7 *Bromination of organic substrates mediated by compound 7.1 or 7.2*

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, $-CH_3$	40
		7.7-7.4(m, 3H), 3.64(s, 3H, $-OCH_3$), 1.1 (s, 3H, $-CH_3$)	20
o-Aminophenol		8.3 (s, 1H), 7.8(d, 1H, J=6Hz), 7.1(d, 1H, J=6Hz),6.2-6.5 (br, 2H, $-NH_2$)	42
m-Aminophenol		7.8-7.25 (m, 3H),5.3-5.0 (br, 2H, $-NH_2$)	39
		8.1-7.5 (m, 3H), 5.6-5.3 (br, 2H, $-NH_2$)	11
p-Aminophenol		8.3 (s, 1H), 7.4(d, 1H, J=6Hz), 6.9(d, 1H, J=6Hz), 6.2-5.9 (br, 2H, $-NH_2$)	43
Quinol		8.5 (s, 1H), 8.2-7.5 (m, 3H)	30

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, $-NH_2$)	35
		7.5-6.36 (m, 4H), 4.0 (s, 2H, $-NH_2$)	23
o-Nitroaniline		8.3 (s, 1H), 7.45 (d, 1H, J=7Hz), 6.75 (d, 1H, J=7Hz), 6.35-6.05 (br, 2H, $-NH_2$)	50
		7.3-7.05 (m, 3H), 6.5 (s, 2H, $-NH_2$)	20
m-Nitroaniline		7.7-7.25 (m, 3H), 4.6-5.0 (br, 2H, $-NH_2$)	40
		7.5-6.7 (m, 3H), 4.5-4.8 (br, 2H, $-NH_2$)	22
p-Nitroaniline		8.25 (s, 1H), 7.9(d, 1H, J=6Hz), 6.64 (d, 1H, J=6Hz), 4.8-4.6 (br, 2H, $-NH_2$)	71

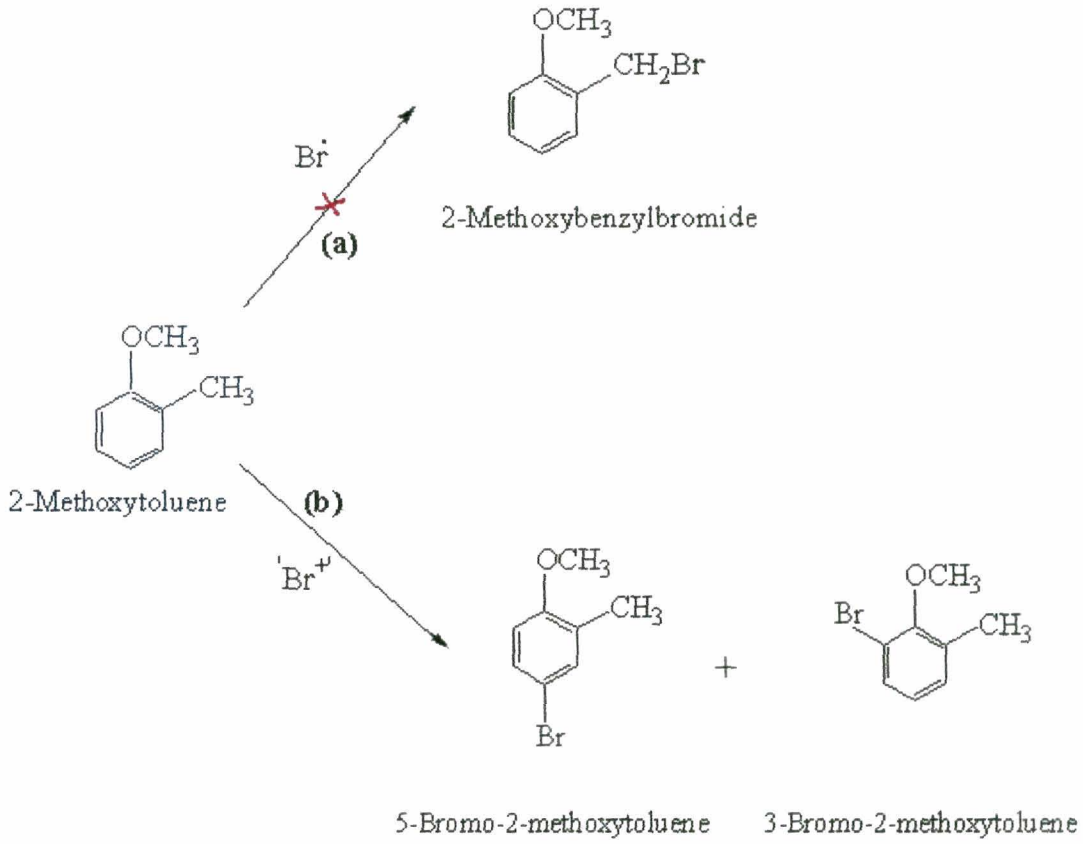


Fig. 7.7 Bromination reaction of 2-methoxytoluene. **(a)** Possible product of bromination through radical reaction, **(b)** electrophilic bromination involving 'Br^{+}' forms exclusively ring substituted products. Bromination reaction using dimeric compound **7.1** or **7.2** produces exclusively bromomethoxytoluene.

occur in their monodentate form while the third one forms a bridge between the metal centers through carboxylate group. Reports are available in the literature on complexes, some of which were structurally characterized, where more than one amino acid or peptide ligand bind the same metal center by exercising different co-ordination modes[27,28]. As rationalized in Chapter 5, such co-ordination are possible because of the presence of several donor sites in these ligands which make them co-ordinatively versatile and susceptible to act as mono-, bi-, or tridentate, or bridging ligands depending on reaction condition and nature of the metal[27-30]. The low pH of the reaction medium appears to be responsible for the co-ordination of the amino acids asparagine and glutamine to the V(V) centre in their zwitterionic form thereby stabilizing the $V_2O_2(O_2)_3$ moiety and enabling their isolation into solid state.

It is notable that, peroxo-vanadium compounds having a bridging peroxo moiety, bonded in a μ-η^1: η^1 fashion, could so far be synthesized only in presence of amino acids and peptides[14,15]. It is reasonable to expect enhanced stability of the vanadium dimer through hydrogen bond interaction with amide groups of the side chain in these ligands. Ready loss of hydrogen bonds on dissolution of the compounds in water indeed is the likely cause of their instability in solution which results in break up of the μ-peroxo vanadate moiety with subsequent release of oxygen.

The side-on bound peroxo groups present in the degradation products, DPV and MPV as seen in the ^{51}V-NMR and electronic spectra, are responsible for the catalase-dependent oxygen release from the compounds. Since the diperoxovanadium complexes **7.3** and **7.4** contain side-on bound peroxo groups they appear to be relatively much more stable in solution and degrade steadily under the action of catalase releasing the

amount of oxygen corresponding to two peroxo groups per molecule in consistence with their formulae.

Experiments of bromide oxidation ability of two types of complexes reveal that only the complexes **7.1** and **7.2** containing μ-peroxo group are capable of bromide oxidation at physiological pH. Obviously the activity of the original compound in water is limited to a short period before its degradation. The results of the studies on the bromination activity of the compounds **7.1** and **7.2** fit well with the proposed scheme of reactions shown in *Fig.6. 8*. Ability of the peroxo-bridged compounds to mediate organic bromination is evident from the studies.

To sum up this investigation concerning the synthesis and some aspects of the chemistry of dinuclear and mononuclear peroxo-vanadate complexes, we would like to emphasize that the dimeric complexes with a μ-peroxovanadate moiety are distinctly different in nature, stability and reactivity from the monomeric anionic complexes possessing exclusively chelated bidentate peroxo-groups. The heteroligand plays an important role in imparting stability to the VOOV group permitting its isolation into solid state. Present experiments confirm the reactivity of the synthetic μ-peroxovanadate dimer in producing bromination competent intermediate. The proposed reaction pathway lends further support for the involvement of a VOOV group in oxidative bromination at physiological pH.

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CHAPTER 8

Summary and Conclusions

Summary and Conclusions

Peroxo-vanadium complexes and their chemistry are pertinent to the reactions catalysed by bromo- and haloperoxidases, in which vanadium cofactor is likely to be associated with a peroxo group during catalysis[1]. In addition, as mentioned in Chapter 1, vanadium and its peroxo compounds have distinct effect on several biological processes[2]. To get a better understanding of the mechanism of vanadium haloperoxidase many vanadium(V) compounds have been prepared and studied as structural and functional enzyme mimics[1,3-5]. Moreover, in order to address the role of V-H_2O_2 system in inhibition or activation of enzyme functions[6,7] intense biological work[1,2,6-10] and solution studies[11-15] have been carried out on interaction of peroxovanadates with biogenic species such as amino acids, peptides and proteins[16-18]. Since very few reports are available on synthetic peroxo-vanadium compounds with co-ordinated peptides[19,20] and information pertaining to dinuclear peroxovanadates are limited[3,21-23], as part of the present research programme, we have directed our efforts to synthesize a series of novel oxo and peroxo-bridged peroxovanadates stabilized by biogenic or bio-relevant co-ligands. Some of the key properties of the compounds such as their stability towards decomposition and activity in various redox processes including biomimetic bromination reaction have been examined.

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

8.1 Synthesis and studies on novel oxo-bridged dinuclear peroxo vanadium(V) compounds

(i) It is possible to isolate the species formed in a solution of diperoxovanadate and VO^{2+} in presence of EDTA which probably corresponds to the intermediate responsible for the EDTA-induced inhibition of DPV-V(IV) mediated redox processes. The reaction led to the synthesis of heretofore unreported dinuclear monoperoxovanadates(V), $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)].2H_2O$, A=Na or K (**3.1** and **3.2**).

(ii) Potential of the above-mentioned reaction to serve as a paradigm for the synthesis of other stable dinuclear monoperoxovanadates, if conducted in presence of suitable ligand was evident when similar reactions carried out by employing NTA or gly-gly as ligand in lieu of EDTA afforded the complexes, $Na_6[V_2O_3(O_2)(NTA)_2(SO_4)(H_2O)].2H_2O$ (**4.1**) and $Na_2[V_2O_3(O_2)(gly\text{-}gly)_2(SO_4)(H_2O)].2H_2O$ (**4.2**). Although SO_4^{2-} is not directly co-ordinated to the peroxovanadate center, these complexes are possibly the only known peroxovanadate compounds containing co-ordinated sulphate.

(iii) Compounds **3.1**, **3.2**, **4.1** and **4.2** are stable in solution at physiological pH, are inactive in NADH or bromide oxidation, and show remarkable resistance to catalase action.

These compounds represent a set of water-soluble dinuclear peroxo derivatives which contain molecules familiar to bioenvironment as heteroligands. Their properties such as high stability in solution at neutral pH and resistance to catalase are expected to be of physiological importance. The heteroligands forming chelate rings might impart this extra stability to the molecules. This may be relevant in the cellular milieu where H_2O_2 has little chance to survive abundant catalase and glutathione peroxidase. By forming peroxo complexes of the above type vanadate may provide a way of preserving cellular H_2O_2 in presence of abundant catalase and make it available for its functions. Although the observed stability of the peroxovanadium 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 biomimetic and therapeutic agent and provide future scope for testing such properties.

8.2 Synthesis and studies on peroxo-bridged dinuclear peroxovanadates

(i) Synthesis of dinuclear peroxovanadate compounds $[V_2O_2(O_2)_3L_3].H_2O$ (L = amino acids or dipeptides) and $[V_2O_2(O_2)_3(\text{tripeptide})_2].H_2O$ with μ-η^1:η^1 peroxo group can be achieved by stabilizing the species formed in a solution of vanadium pentoxide and H_2O_2 at highly acidic pH, in presence of suitable amino acids or peptides under appropriate reaction conditions. The ligands seem to stabilize the peroxo-bridge by inter-ligand interaction, possibly hydrogen bonding.

Isolation of monomeric products $Na[VO(O_2)_2L].H_2O$, L= asparagine (**7.3**) and glutamine (**7.4**), from similar reactions conducted at relatively higher

pH underscore the importance of pH in achieving desired synthesis of hetero-ligand peroxovanadium compounds.

(ii) Peroxo-bridged compounds undergo rapid degradation on dissolving in water with partial loss of peroxide accompanied by release of oxygen. The ^{51}V-NMR spectra of such solutions showed diperoxovanadate and decavanadate as major degradation products. Additional oxygen release takes place on treating these solutions with catalase as expected of residual diperoxovanadates.

(iii) Dinuclear compounds (**5.1**, **5.2**, **5.3**, **5.4**, **7.1**, **7.2**) when directly added to aqueous reaction solution instantaneously oxidized bromide to a bromination competent intermediate at physiological pH. Instant bromination activities of the original compounds were limited to a transient period and lasted until breaking up of the peroxo bridged species.

After the initial fast bromination activity, the degradation products formed in solution viz., DPV and vanadate together gave the slow secondary bromination.

(iv) Peroxo-bridged divanadate compounds are also capable of mediating bromination of aromatic substrates in aqueous - organic media.

(v) Dimeric compounds with μ-η^1:η^1 peroxo moiety are distinctly different in nature, stability and reactivity from the monomeric anionic complexes with similar co-ligands, but possessing exclusively chelated bidentate peroxo-groups.

The monomeric diperoxovanadate compounds are relatively more stable in solution, degrade steadily under the effect of catalase, and are inefficient in bromination at physiological pH.

The informations derived from the above studies support the proposal that an active group present in the peroxo-bridged complexes is responsible for the ready oxidation of bromide and subsequent bromination of substrate. The proposed reaction pathway confers the status of a selective bromide oxidant, at physiological pH, on VOOV group. These findings make the dinuclear complexes possible candidates of mimic in the action of vanadium in bromoperoxidase. However, it is not completely clear as to why a peroxo-bridged divanadate moiety is much more reactive in oxidative bromination than a peroxo group bonded to V(V) in a side-on fashion. Theoretical computations are expected to provide valuable insights into this aspect and such studies are being initiated.

We are constrained in concluding that any of the reactions of the proposed reaction scheme (*Fig. 6.8*) are involved in the action of the enzymes in view of lack of direct evidence. However, since crystal structures of haloperoxidase proteins *Ascophyllum nodosum*[24] and *Corallina officinalis*[25] showed dimeric subunits with a vanadium per subunit, the possibility of the active site in the protein having a vanadium dimer can not be ruled out. Informations generated from the present investigation may find relevance in the context of designing safer biomimetic redox catalysts for organic bromination which constitute an active area of current research[26-28].

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APPENDIX

List of Publications

List of publications

Papers published / accepted for publication:

1. *A dinuclear peroxo-vanadium(V) complex with coordinated tripeptide. Synthesis, spectra and reactivity in bromoperoxidation*

   S. Sarmah and N. S. Islam, *J. Chem. Res.(M)*, 2001, 0536; *J. Chem. Res.(S)*, 2001, 172.

2. *Reaction of diperoxovanadate with vanadyl sulphate in presence of EDTA as an access to dinuclear peroxovanadates(V)*

   S. Sarmah, P. Hazarika and N. S. Islam, *Polyhedron*, 2002, **21**, 389.

3. *Peroxo-bridged divanadate as a selective bromide oxidant in bromoperoxidation*

   S. Sarmah, P. Hazarika, N. S. Islam, A. V. S. Rao and T. Ramasarma, *Mol. Cell. Biochem.*, 2002, **236**, 95.

4. *Synthesis of newer dinuclear and mononuclear peroxo-vanadium(V) complexes containing biogenic co-ligands. A comparative study of some of their properties*

   S. Sarmah, D. Kalita, P. Hazarika, R. Borah and N. S. Islam, *Polyhedron* (accepted for publication).

Table 7 Total / partial energies of 2,4-dimethyl-6-oxo-2,4-heptadienonitriles 2 – 5

Isomer	Conformer	Total energy	Angle strain	Torsion	Non 1,4 VDW	dipole\dipole
2 (2 *Z*,4 *Z*)	*s-cis s-cis*	7.06	1.85	4.12	0.15	2.99
	s-cis s-trans	10.56	3.39	5.04	1.22	0.42
	s-trans s-cis	8.70	4.57	2.09	3.20	1.49
	s-trans s-trans	11.76	7.94	2.96	2.92	0
3 (2 *Z*,4 *E*)	*s-cis s-cis*	6.07	2.26	0.09	1.17	1.68
	s-cis s-trans	8.64	3.81	0.67	0.80	0.77
	s-trans s-cis	6.77	2.91	1.39	1.76	1.15
	s-trans s-trans	7.99	4.90	1.69	1.23	1.09
4 (2 *E*,4 *Z*)	*s-cis s-cis*	8.25	2.37	4.27	0.47	1.86
	s-cis s-trans	10.09	3.33	4.64	0.92	-0.66
	s-trans s-cis	8.51	3.59	0.04	1.47	1.22
	s-trans s-trans	9.62	4.53	0.89	0.49	0.77
5 (2 *E*,4 *E*)	*s-cis s-cis*	7.93	4.31	1.23	1.26	1.14
	s-cis s-trans	8.88	5.06	0.40	0.13	1.00
	s-trans s-cis	6.77	3.56	1.48	1.04	1.36
	s-trans s-trans	8.72	5.21	1.61	1.00	0.78

Similar MM2 calculations were performed for the α pyranic diastereoisomeric intermediates or transition states T_1 and T_2. Geometrical and energy characteristics of these two structures are presented in Table 8: total energies are practically equal but T_1 is less puckered than T_2, suggesting that it may be preferred.

Table 8 Energies (kcal/mol) of α pyranic isomers T_1 and T_2 and dihedral angles (degrees)

		T_1	T_2
Energy	total energy	10.24	10.22
	angle strain	2.69	2.65
	torsion	1.58	1.56
	non 1,4 VDW	2.01	2.02
	dipole\dipole	3.99	3.97
Dihedral angle	O(5) C(3) C(4) C(9)	3.62	4.51
	C(4) C(3) O(5)-C(7)	3.72	5.11
	C(3) C(4) C(9) C(8)	1.31	1.18
	C(3) O(5) C(7) C(8)	1.82	2.79
	O(5) C(7) C(8)-C(9)	0.71	0.78
	C(7) C(8)-C(9) C(4)	0.95	1.55

Experimental

Melting points were determined on a Boetius hot plate and are uncorrected. The IR spectra were recorded on a Carl Zeiss UR 20 instrument. The NMR spectra were recorded with a Varian Gemini 300 BB instrument operating at 300 MHz for ^1H and at 75 MHz for ^{13}C.

Reaction of pyrylium perchlorates with sodium cyanide. Synthesis of isomers 2, 4, 7, 10, 11

The pyrylium salt (0.01 mol) was shaken for 10–15 min with 2% aqueous sodium cyanide (0.012 mol) solution in the presence of ethyl ether. The upper layer was separated, washed with saturated sodium chloride solution, dried over anhydrous sodium sulphate, and the solvent was evaporated under reduced pressure.

The stereoisomers 2–4 were separated by column cromatography on silica gel eluting with a mixture of petroleum ether with ethyl ether in ratios from 20:1 to 1:1 (v/v); the first compound to be eluted was 2 (2 *Z*,4 *Z*) isomer.

The global yield (2+4) for each batch is 92% or higher.

Synthesis of 4 E isomers

Compounds 2, 4 or 7 were converted into the 4 *E* isomers by treatment with an excess of 35% hydrochloric acid at room temperature. After 5 min the reaction mixture was diluted with water and extracted with ethyl ether or was cooled and filtered off in the case of compound 3.

Heptadienonitrile 3

m. p. = 42–44°C (lit.[2] m. p. = 50°C).

(2 Z,4 Z) 7-(1-Methylethyl)-4,7-dimethyl-6-oxo-octadienonitrile 7

IR (CCl_4): 1683 (CO), 2222 (CN).

^1H NMR ($CDCl_3$): 2 *i*Pr: 1.21 (6H, d, J=6.9Hz), 2.65 (1H, sep d, J_1=6.9Hz, J_2=1.0Hz), 6 *i*Pr: 1.11 (6H, d, J=6.9Hz), 2.66 (1H, sep, J=6.9Hz), 4 Me: 2.37 (3H, d, J=1.6Hz), 3 H: 7.77 (1H, dd, J_1=1.2Hz, J_2=1.0Hz), 5 H: 6.25 (1H, q, J=1.4Hz).

^{13}C NMR($CDCl_3$): 2 *i*Pr: 21.2 (CH_3), 35.3 (CH), 6 *i*Pr: 18.0 (CH_3), 41.7 (CH), 4 Me: 22.8, Cq: 117.4 (CN), 133.1 (2 C), 146.7 (4 C), 204.2 (CO), CH: 139.7 (3 CH), 128.2 (5 CH).

(2 Z,4 Z) 7-(1-Methylethyl)-4,7-dimethyl-6-oxo-octadienonitrile 8

^1H NMR ($CDCl_3$): 2 *i*Pr: 1.21 (6H, d, J=6.8Hz), 2.61 (1H, sep d, J=6.9Hz, J=1.2Hz), 6-*i*Pr: 1.13 (6H, d, J=6.8Hz), 2.08 (1H, sep, J=6.8Hz), 4 Me: 2.40 (3H, d, J=1.3Hz), 5 H: 6.45 (1H, q, J=1.2Hz), 3 H: 6.51 (1H, dd, J_1=J_2=1.2Hz).

^{13}C NMR($CDCl_3$): 2 *i*Pr: 21.2 (CH_3), 35.4 (CH), 6-*i*Pr: 18.0 (CH_3), 42.1 (CH), 4 Me: 16.9, Cq: 116.9 (CN), 122.5 (2 C), 147.0 (4 C), 204.9 (CO), CH: 128.4 (5 CH), 143.9 (3 CH).

(2 Z,4 E) 2(1 thi) 4,6-dimethyl 6 oxo heptadienonitrile 10a

IR (CCl_4): 1691(CO), 2221 (CN).

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A dinuclear peroxo vanadium(V) complex with a coordinated Tripeptide. Synthesis, spectra and reactivity in Bromoperoxidation

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The triglycine peroxo complex $(V_2O_3(O_2)_2(Gly\ Gly\ Gly)]\cdot H_2O$ has been synthesised from the reaction of V_2O_5 with H_2O_2 and triglycine at pH 2. The compound has been characterised by elemental analysis and spectral studies. In the dimeric molecular complex the two vanadium centres are bridged by a peroxide group. The triglycine, occurring as a zwitterion, binds the vanadium by O (carboxylate) and O (amide) atoms. The complex oxidises bromide to give a bromination competent intermediate in phosphate buffer at physiological pH. Addition of its solid to bromide solution instantly converted phenol red to its 592nm absorbing bromo derivative. The high bromination activity was lost on dissolving the compound in water with partial loss of peroxide. Diperoxovanadate and decavanadate were found to be the products by ^{51}V NMR spectrum. Further release of oxygen was observed on treating this solution with catalase. The results indicate that peroxo-bridged divanadate is the active intermediate in the vanadium catalysed bromoperoxidation reaction.

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Introduction

Vanadium is a trace metal that influences a variety of biological functions [1-3] The recent findings on the role of vanadium in bio processes include its presence as an essential constituent of some proteins e g bromoperoxidase [1] its inhibition of hydrolysis of phosphoproteins [4] and capability of its peroxo complexes to mimic actions of insulin[5] and oxidise NADH[6] and bromide [7]

Bromoperoxidase involved in the biosynthesis of a variety of naturally occurring brominated products is the first enzyme shown to contain protein bound vanadium that is essential for its activity [1,2] Functional mimics of vanadium bromoperoxidase have been described for bromination of selected organic compounds [8-11] Oxidation of bromide by H_2O_2 to hypobromous acid capable of transferring a bromine atom to an acceptor molecule occurs in acidic medium at pH<3 0 Extremely slow at pH>5 0 this reaction is catalysed by vanadium compounds such as $VOSO_4$[8] and V_2O_5 [9,10] The most probable oxidants of bromide monoperoxovanadate (MPV) and diperoxovanadate (DPV) are readily formed on adding excess H_2O_2 to vanadate solution [12-15] with DPV predominating at pH>5 0 However synthetic DPV and also MPV could not substitute for the V_2O_5 and H_2O_2 mixture in the bromination process [14] It was then proposed that a dioxotriperoxodivandate complex presumed to form by a combination of MPV and DPV is the active oxidant [15] but this vanadium dimer was found only in highly acidic medium (pH<3 0) and with high concentrations of vanadate and H_2O_2 The underlying need for the presence of vanadyl[1] or excess vanadate[10] for effective bromination in the early experiments remained unexplained

A notable feature of the DPV dependent bromination at pH>5 was the requirement of vanadyl (V^{IV}) or vanadate (V^{V}) [16] This implied that the inactive DPV gains oxidant activity by complexing with vanadyl A μ peroxo bridged divandate intermediate $[OVOOV(O_2)]^3$ was proposed as a proximate oxidant of bromide[16] as well as of NADH [16] Support for such an intermediate as the bromide oxidant came from studies on a synthetic compound with a VOOV bridge [20] $\{V_2O_2(O_2)_3(GlyH)_2(H_2O)_2\}$ which could produce a bromination competent intermediate in phosphate buffer and physiological pH [21] With an aim to provide further evidence in confirmation of this

potential of bromide oxidation and also in view of the paucity of information on peroxo bridged divanadate species we were interested in synthesising newer members of such complexes stabilised by biogenic heteroligands viz. di and tripeptides Peptides are probably the primary ligands to interact with vanadate and vanadyl in biological systems A better understanding of the complexation behaviour of vanadium with such ligands is therefore of vital interest and has been studied in recent years[22-26] However reports related to synthesis and characterisation of peptide peroxovandate complexes are limited [26,27] We report here synthesis of a novel dinuclear tripeptide peroxovanadate with a bridging peroxide group and its activity in bromoperoxidation of organic substrate

Experimental

The chemicals used were all reagent grade products The triglycine and catalase were obtained from Sigma Aldrich Chemical Company

Synthesis of $[V_2O_2(O_2)_3(Gly\text{-}Gly\text{-}Gly)_2]\,H_2O$

V_2O_5 (0 25g 1 37 mmol) was mixed with Gly Gly Gly (0 76g 4 02mmol) in a 250 ml beaker maintaining the molar ratio of V peptide at 1 1 5 To this 30% H_2O_2 (15 ml 132 3 mmol) was added gradually with constant stirring The reaction mixture was stirred for c 15 minutes in an ice bath keeping the temperature below 10°C till all solid was dissolved A red coloured solution of pH 2 resulted at this stage No attempt was made to adjust the pH On adding pre-cooled ethanol (c 50ml) to the above solution under continuous stirring an orange coloured pasty mass separated After standing for c 15 minutes in the ice bath the supernatent liquid was decanted and the residue was treated repeatedly with acetone ethanol mixture under scratching until it became a micro crystalline solid The product was separated by centrifugation washed with ethanol and dried in vacuo over conc sulfuric acid

Anal Calc for $\{V_2O_2(O_2)_3(Gly\ Gly\ Gly)_2\}\,H_2O$ V 16 29 O_2^2 15 34 C 23 0 H 3 51 N 13 42 Found V 16 6 O_2^2 15 25 C 23 4 H 4 0 N 13 9 Yield approximately 57%

The compound in the solid state was found to be stable for several weeks stored dry below 20°C but tended to be hygroscopic at ambient conditions and decompose in few days

Spectroscopic Measurements and Analysis

IR spectra were recorded on a Nicolet Model Impact 410 FTIR spectrometer with the samples as KBr pellets Electronic absorption spectra were recorded on a Hitachi Model 2001 spectrophotometer All the absorbance values are denoted as e g A_{592} A_{540} at the wavelengths indicated The ^{51}V NMR spectra were recorded in a Brucker AMX 400FT spectrometer at vanadium frequency of 105 190 MHz with the sample in a 10mm spinning tube with a sealed co axial tube containing D_2O which provided the lock signal The chemical shift data are shown as negative values of ppm with reference to $VOCl_3$ at 293 K

Vanadium was determined volumetrically by titration with potassium permanganate The total peroxide content was determined by adding a weighed amount of the compound to a cold solution of sulfuric acid (1M 100ml) containing 1 5g of boric acid and titration with standard potassium permanganate or with standard Ce(IV) solution [28] The compound was analysed for C H and N at R S I C North Eastern Hill University Shillong and at the Department of Organic Chemistry IISC Bangalore India

Measurement of bromination activity

The method of de Boer et al[29] of introducing four bromine atoms into the molecule of phenol red to form bromophenol blue was used as a measure of bromination activity Phenol red acts as an efficient trap of active bromine species without influencing the rate of the reaction until it is exhausted The reaction mixture contained phosphate buffer (50mM pH 5 5) KBr (2M) and phenol red (20μM) The reaction was started by adding a weighed amount (3 5mg) of the solid compound and was monitored at 30°C by the increase in absorbance at 592nm of bromophenol blue (ϵ = 67 4) The volume of the reaction mixture was kept at 25ml and aliquots were transferred to the spectrophotometer

immediately after mixing to record the jump in A_{592} (instant change) and the steady rate of increase that followed was also calculated

Instant bromination activity	65μM bromine transfer per mM compound
Secondary rate of bromination	8μM Br transfer/min with 1 mM compound

Measurement of catalase dependent oxygen release

A Gilson 5/6 H oxygraph fitted with a Clark oxygen electrode was used for measuring changes in the concentration of dissolved oxygen in the medium (0 224mM at 30°C) The rate and total amount of oxygen released from 0 2mM solution of the compound in phosphate buffer (50mM pH 7 0) on adding catalase (0 08mg protein/ ml) were measured The changes in oxygen concentration are shown as units of μM

Results and discussion

The importance of pH for the successful synthesis of peroxo metal compounds has been emphasised in the literature [12-28] One of the essential parameters for achieving success of the synthesis of the peroxo-bridged complex $\{V_2O_2(O_2)_3(Gly\ Gly\ Gly)_2\}\,H_2O$ was the use of acidic medium The pH value of 2 attained spontaneously during the reaction was not raised The use of alkali and hence the presence of counter cations in solution was thus avoided The preferred mode of co ordination of a peptide is also dependent on the pH of the reaction medium and the nature of the metal [22-24,30-33] A simple peptide can act as a mono bi or tridentate ligand with different combinations of binding sites viz terminal amino carboxylate and amide groups and can occur in complexes in either neutral zwitterionic[24,31] or anionic forms [23,26,31-33] In the present case the low pH of the reaction medium probably favoured the co-ordination of the triglycine to V(V) in its zwitterionic form thereby stabilising the $V_2O_2(O_2)_3$ moiety and leading to the synthesis of the desired molecular complex

The data on elemental analysis gave crucial information on the composition of the compound A ratio of 2 3 was obtained for V peroxide which suggested a dimeric nature of the complex, presumably involving a bridging peroxide group The vanadium triglycine ratio was ascertained to be 1 1

The significant features of the IR spectrum of the newly synthesised tripeptide peroxo vanadate complex involve absorptions due to ν(V=O) co-ordinated peroxide co ordinated peptide and lattice water The spectrum enabled clear identification of ν(V=O) at 952cm^{-1} arising from a terminally bonded V=O group[34,35] A peroxo group bonded terminally to the V(V) center in a chelated fashion exhibits a strong ν(O-O) band in the 880 870cm^{-1} region [20,35] Appearance of two ν(O-O) bands in the complex, one at 845cm^{-1} and another at a lower frequency of 805cm^{-1} with some broadening indicated the presence of two structurally different peroxo groups the terminal chelated and the bridging type Similar observations were made previously in the IR spectra of peroxo vanadate complexes possessing a peroxo bridge and amino acids as heteroligands [20,36] The bands at 619 and 560cm^{-1} have been assigned to ν_2 and ν_3 modes of V O_2 vibrations [20,35]

In the IR spectrum of the complex the pattern originating from co ordinated peptide showed N H stretching bands in the 3255 to 3083cm^{-1} region as expected from the –N H_3 group Significant differences between the IR spectral pattern of the triglycine complex and that of the free triglycine were observed in the region 1685 1600cm^{-1} Three distinct bands were observed for the complex at 1678cm^{-1} 1610cm^{-1} and 1389cm^{-1} which were assigned to ν(C=O) (amide I) ν_{as}(COO) and ν_s(COO) modes of co ordinated triglycine[37,39(b)] respectively In the spectrum of the free ligand the antisymmetric and symmetric vibrations of the carboxylate group occur[39(a)] at 1598 and 1405cm^{-1} These changes in the frequency of decrease in ν_s(COO) and increase in ν_{as}(COO) are typical of carboxylate group co ordinated in an unidentate zwitterionic fashion [39(a)] The small shift of the ν(C=O) (amide) to a lower frequency with some broadening as compared to free triglycine (1684cm^{-1}) was probably due to the participation of one of the carbonyl groups in co ordination [3] Involvement of N(amide) in co ordination is known to cause considerable decrease in the peptide carbonyl stretching frequency [32,39(b)] The broadening of the band at 1678cm^{-1} was probably due to the presence of both co-ordinated and free amide groups in the complex Other characteristics of the spectrum were the observance of the N–H deformation modes due to the amide and N^+H_3 groups in the 1600–1520cm^{-1} region and the indication of the presence of lattice water Based on these observations it may be inferred that the triglycine ligands occurring as zwitterion co-ordinate to the vanadium(V) through O(carboxylate) Co-ordination of one of the carbonyl (amide) groups of the peptide chain probably completes the hexaco-ordination of vanadium in the complex leading to the formation of a seven membered ring around each vanadium see ref 24) A provisional structure of the type shown in *Fig* 1 has been envisaged for the complex The second amide group in the peptide side chain is not shown in the structure for simplicity It is possible that hydrogen bonding between the peptide side chains stabilise the molecule

On adding the solid compound to water bubbles of oxygen gas were observed to be released in solution for about 30 min Oxygen was confirmed to be the liberated gas in the oxygraph Instability of the complex in water and degradative loss of the peroxide groups were implicit The electronic spectrum of the compound recorded after bubbles ceased displayed a single broad LMCT band at 328nm (ε=720) On adding the enzyme catalase to the solution further dioxygen release took place A maximum of 1 5 O_2 per mole of the compound will be released by the catalase if all the three peroxides were retained A lower experimental value of 0 6 was realized for the compound since a part of the peroxide was initially lost as dioxygen The rate of dioxygen release (12 1μm/ min) paralleled the residual peroxide concentration Addition of EDTA (1mM) decreased the rate to about 10% The inhibition is probably due to the complex formation of EDTA with the peroxo vanadate species which is resistant to catalase action

In order to derive further information regarding the nature of the complex in solution it was subjected to ^{51}V NMR studies Based on the available data[42,44] assignments were made to the peaks observed in the spectrum The peaks at –424 –509 and –525 ppm correspond to decavanadate formed as a result of depletion of the peroxovanadate in the acidic solution The signal at –694 ppm indicated the formation of DPV The two resonances observed near –714 ppm were probably due to vanadium with co ordination of the peptide through carboxylate oxygen [23] There may be small variations in the observed chemical shift values from those reported owing to the ligand being still co-ordinated to some of the products and to variations in pH Thus from the NMR studies it was apparent that the complex undergoes rapid degradation in water and no direct information regarding the nature of the original solid compound could be

obtained Addition of EDTA an inhibitor of the dioxygen release reaction to the solution of the complex and recording its spectrum showed a small peak at –762 ppm A compound showing a chemical shift at –760 ppm was previously found in the mixture of vanadate and H_2O_2 with phenylalanyl glutamine [41] and was suggested to be due to a dimeric vanadate Therefore the possibility of –760 ppm peak originating from the undegraded original dinuclear vandate cannot be ruled out

The redox activity of the triglycine perexo vanadate compound was investigated by adding a weighed amount of the solid to a standard reaction mixture of bromide in phosphate buffer with phenol red as the trap for oxidised bromine The colour of the solution instantly turned blue and the spectrum recorded showed three major changes a peak at 237nm due to a mixture of vanadate compounds decrease in A_{237} due to loss of phenol red a peak at 592nm characteristic of the product bromophenol blue (*Fig* 2 a) After the instant activity a slow increase in A_{592} (*Fig* 2 b) indicated a secondary rate (ΔA_{592}/min = 0 03/min) of bromination and the product increased in amount as seen by the increase in the peak A_{592} (*Fig* 2 c)

In a similar reaction with phenol red omitted a large peak at 262nm with a shoulder at 237nm was observed on adding the solid complex Addition of phenol red to this solution resulted in the decrease in A_{262} and a predominant peak at 592nm appeared indicating the formation of bromophenol blue The 262nm peak therefore represents a bromination competent oxidised species of bromide possibly an equilibrium mixture of BrOH Br_2 and Br_3^- as proposed earlier [42]

The foregoing experiments show that the triglycine peroxovanadate compound has the instant and secondary bromination activity The fast reaction occurs giving a large increase in A_{592} Of the expected bromination 16% was realised as instant activity The results imply that the complex a peroxo-bridged dimer must possess an active group that readily oxidises bromide The moment the compound is added to water the instant activity is lost due to the parallel loss of the active group It is obvious that the degradation products formed in solution viz DPV and vanadate together give a secondary reaction rate of bromination Under these conditions DPV (1mM) was completely inactive and in the presence of vanadate (0 2mM) showed the expected secondary rate but not the instant activity The need for peroxo-bridged divandate to oxidise bromide is thus apparent

The redox Chemistry of vanadyl vanadate and peroxovanadates described earlier by Brooks and Sicilio[13] Jaswal and Tracey[15] and Ramasarma et al[18,21,44] allow us to explain the reactions involved in the present study We suggest that in this highly reactive compound the triglycine ligands stabilise the μ peroxo bridge between the two peroxovanadate moieties through hydrogen bonding formed between the peptide side chains leading to its isolation in the solid state Thus the hydrogen bonding between the two peptide ligands is probably responsible for providing the molecule with distinctive structure of a peroxo bridge and high bromination activity The moment the compound is added to water the hydrogen bonding weakens the dimer separates and the instant bromination activity vanishes These observations strengthen the earlier proposal[21,44] that VOOV is the active group in the vanadate dependent bromination activity and is the likely primary oxidant of bromide in phosphate buffered medium yeilding a bromination competent intermediate $BrOVO(O_2)$ that can transfer the bromine atom to acceptor AH[21,18] (reactions a and b in *Fig* 3) The secondary bromination activity depends on DPV and vanadate formed from the complex in solution (*Fig* 3 reaction c) It is generally agreed that DPV alone is incapable of supporting bromination [16,17,44] Dismutation of MPV generates vanadate which is then reduced to vanadyl by bromide and acid medium[18] (reactions c d) Vanadyl complexes with DPV to form a μ peroxo-bridged vanadate dimer (reaction f) [13,18] This complex can then oxidise bromide and produce a bromination competent intermediate (similar to reactions a and b) that can be recycled till phenol red is exhausted The reactions e – h shown in *Fig* 3 indicate the way dioxygen is released when the solid compound was added to water in absence of bromide The process involves interaction of DPV with vanadyl formed from vanadate in acid medium and degradation of the μ peroxo complex [18] Formation and decay (reactions g and h) of oxygen radical species of peroxovanadate are implicated in this process as described earlier [13,18,21,44] The striking feature of the essentiality of vanadyl for making an effective bromide oxidant from DPV was thus accomodated as a $V^{IV}OO\,V^{V}$ intermediate in the reaction pathway in *Fig* 3

In summary we have demonstrated that it is possible to isolate the dioxo triperoxo vanadate species in the solid state through complexation with amino acids[20,36] and peptide The occurrence of the triglycine as a zwitterion in the complex and its mode of co ordination as a bis(ligand) involving O(carboxylate) and O(amide) is different from that in solid glycylglycine monoperoxovanadate complex [76] as well as peptide peroxo vanadate systems studied in solution at near neutral pH [22,23] where the preferred mode of peptide co-ordination was found to be tridentate

The studies on the redox activity of the synthetic μ peroxovanadate compound confirm its ability in producing a bromination competent intermediate at physiological pH The results are in support of participation of such VOOV type oxidants in vanadium catalysis It appears possible that the active site in the proteins have a vanadium dimer since crystal structures of two of the enzyme proteins[2,3] showed dimeric subunits with a vanadium atom per subunit We are however restrained to suggest any involvement of *the reactions discussed above in the action of the enzymes mainly because peroxo groups* are not yet located in these enzyme structures But from reports available there appears to be a definite potential of the peroxovanadate intermediates (*Fig* 3) in oxidative modifications in biological systems

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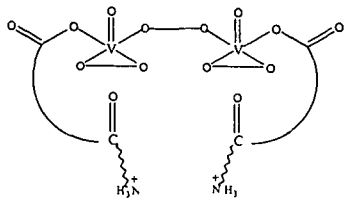

Fig 1 Proposed structure of peroxovanadate compound $\{V_2O_2(O_2)_3(Gly\ Gly\ Gly)_2\}\ H_2O$ The second amide group in the peptide side chain is not shown

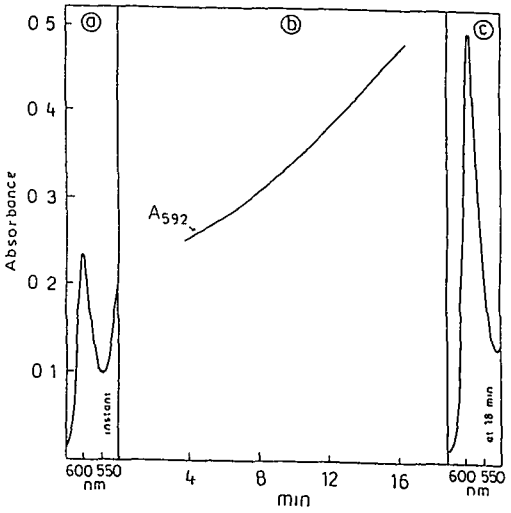

Fig 2 Bromination activity with compound a) spectrum taken immediately after adding the compound (0 14 mg/ml) to the reaction mixture showing the instant activity b) the increase in A_{592} indicating the secondary rate c) the peak at 592 nm at 18 min of the reaction

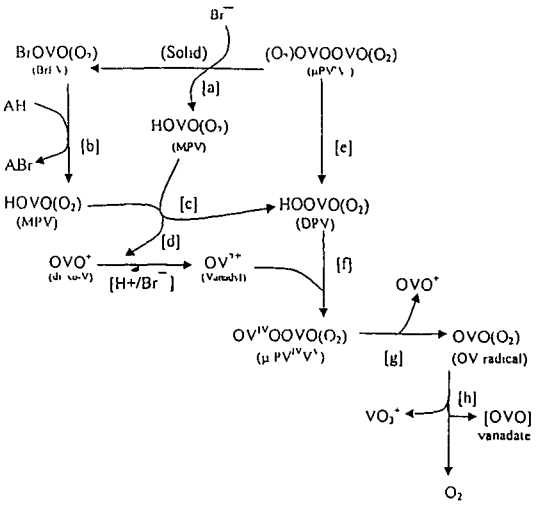

Fig 3 Schematic representation of reactions occurring with peroxo bridged divanadate compounds a) formation of active bromine compound BrPV and MPV when solid is added to a bromide solution b) transfer of bromine to acceptor AH (phenol red) c) dismutation of MPV to DPV and vanadate d) reduction of vanadate to vanadyl by acid and bromide e) seperation of DPV and MPV on adding solid compound to water (in absence of bromide) f)formation of a μ peroxo compound from DPV+vanadyl g) breakdown of the μ peroxo group h) dismutation of OV radical releasing O2 The triglycine ligands in the compound are not shown Valency state of reduced vanadium is shown as VIV and all others are VV No attempt is made to show exact stoichiometry of reactions

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Difunctional heterocycles a convenient synthesis of bis(pyridinyl 2 3 dihydrooxadiazolyl)benzenes

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Bis(pyridinyl 2 3-dihydrooxadiazolyl)benzenes 5a-e and 9a b are obtained in 25 85% yields by heating the corresponding bis(hydrazones) 4a f and 8a d in refluxing Ac O AcOH for 3 5 h while the bishydrazones 19 and 17b give 33 54% yields of the phthalazine derivatives 20a b upon heating in refluxing ethanol containing acetic acid

There is an interest in the chemistry of oxadiazoles on account of their biological properties For example studies have revealed that substituted 1 3 4-oxadiazoles exhibit bactericidal[1] fungicidal[2] antimicrobial[3] antiinflammatory[4] and antiproteolytic properties[5] In addition pyridines are reported to exhibit diverse biological activities as antimycotic[6] antidepressant[7] and antiarrhythmic[8] agents and they also have potential uses as therapeutic agents [9 11] The presence of these two rings in one molecule might combine the biological activities of both moieties

In connection with these findings and in continuation of our recent interest in the synthesis of difunctional building blocks and their use in the synthesis of bisheterocycles[1 14] we report here the synthesis of novel isomeric bis(pyridinyldihydrooxadiazolyl)benzene derivatives During the last decades such types of structure have attracted attention as model compounds for polymers [15 20] and it has also been observed that many biologically active natural and synthetic products have molecular symmetry [21] We also discuss the formation of unexpected products in some reactions

Results and Discussion

Our strategy to synthesize the new bis(3 pyridinyl 2 3 dihydrooxadiazol 5 yl)benzene derivatives 5a e is outlined in Scheme 1 Thus reaction of diethyl isophthalate (1a) and diethyl terephthalate (1b) with hydrazine hydrate in refluxing ethanol afforded the corresponding bishydrazides 2a b[22] respectively Condensation of the latter with the appropriate pyridine carboxaldehydes 3a e in refluxing ethanol afforded 88 95% yields of the corresponding

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Reaction of diperoxovanadate with vanadyl sulphate in presence of EDTA as an access to dinuclear peroxovanadates(V)

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Abstract

The reaction of alkali–metal diperoxovanadate with vanadyl sulphate in the presence of EDTA afforded the dinuclear heteroligand peroxovanadates(V), $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)]\,2H_2O$, A = Na (1) or K (2) The compounds were characterised by elemental analysis, magnetic susceptibility and spectral studies The two vanadium(V) centres in the complex ion are bridged by an oxo group and a hexadentate EDTA ligand One of the vanadium(V) centres of the dinuclear species contains a bidentate peroxide, whereas the other vanadium centre is bonded to a unidentate sulphate and water leading to hepta-co-ordination around each vanadium(V) Oxygen release reactions and molar conductance measurements revealed that the compounds were stable in solution The compounds were resistant to catalase and were unable to oxidise NADH or bromide It is proposed that this dinuclear complex species corresponds to the complex formed in solution responsible for EDTA induced inhibition of oxidation of NADH and bromide by a mixture of diperoxovanadate and vanadyl © 2002 Published by Elsevier Science Ltd

Keywords Diperoxovanadate Vanadyl EDTA inhibition EDTA bridged heteroligand peroxovanadates Catalase resistance Oxo bridged peroxovanadates containing sulphate

1. Introduction

Diperoxovanadate is receiving importance as a biologically active vanadium compound It mimicks insulin action [1] and was found to have antineoplastic activity [2,3] An intermediate derived from diperoxovanadate was shown to stimulate oxidation of NADH [4] It has been reported earlier by others [4–7] that diperoxovanadate (DPV) gains oxidant activity by complexing with vanadyl (V^{IV}) to form a highly reactive peroxo-bridged intermediate, $[OVOOVO(O_2)]^+$, which could act as oxidant species of NADH [4] and bromide [5], as well as inactivate glucose oxidase [6] or release oxygen as gas in the absence of any substrate [7] Our studies on synthetic compounds with a 'VOOV' bridge of the type, $[V_2O_2(O_2)_3L_2]$ (L = amino acid or peptide) lent further support for such an intermediate as the powerful bromide oxidant at physiological pH [8,9]

One interesting finding of such studies was the inhibitory effect of certain organic ligating agents viz EDTA, histidine, imidazole etc on the above redox processes [4–9] Such inhibition was found to be maximum in the presence of EDTA EDTA induced inhibition was also reported to occur in the oxygen release reaction involving H_2O_2 and $VOSO_4$ [10] The basis for such potent inhibition appeared to be the inactivation of the reactive peroxo intermediates by the ligands through complexation with V(V) and V(IV) centres However, there has been a paucity of evidence on the exact identity of the actual species responsible for such an inhibitory effect [4–8]

We therefore, considered it worthwhile to investigate the reaction of DPV and VO^{2+} in presence of EDTA with an aim to ascertain the nature of the above inhibitory complex by attempting to isolate such species in the solid state Moreover, it has been realised that any information related to the interaction of vanadate or vanadyl with ligands like EDTA may be relevant in addressing the role of vanadium in vanadate mediated inhibition or activation of enzymes [11,12] In view of the continued search for stable peroxovanadate com-

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pounds suitable for therapeutic use [13–15], an additional goal of the present study was also to explore the possibility of gaining an access to newer heteroligand peroxovanadate compounds stable at physiological conditions

We report here, an account of the reaction of alkali diperoxovanadate (ADPV) with $VOSO_4$ in presence of EDTA which led to the synthesis of novel dimeric peroxovanadates of the type $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)]\,2H_2O$ thought to correspond to the inhibitor complex in the above redox processes The compounds have been subjected to dissolution and reactivity studies in order to determine their nature and stability in solution

2. Experimental

The chemicals used were all reagent grade products Catalase and NADH were obtained from Sigma–Aldrich Chemical Company The water used for solution preparations was deionised and distilled

2 1 Preparation of alkali metal diperoxovanadate, $A[VO(O_2)_2(H_2O)]$ (A = Na or K)

Alkali metal diperoxovanadate (ADPV) was prepared by adding an equal volume of H_2O_2 solution (40 mM) to a vanadate solution (20 mM) maintaining the pH at 7 0 by adding dilute alkali hydroxide solution On addition of pre-cooled EtOH to this solution, a yellow microcrystalline product precipitated out which was separated by centrifugation, washed with EtOH and finally dried over conc H_2SO_4 Analysis of the content of vanadium and peroxide agreed with the formula $A[VO(O_2)_2(H_2O)]$ (A = Na or K)

2 2 Reaction of $A[VO(O_2)_2(H_2O)]$ (A = Na or K) with $VOSO_4$ in presence of EDTA Synthesis of $A_4[V_2O_3(O_2)\,(EDTA)\,(SO_4)\,(H_2O)]\,2H_2O$

In a typical reaction the disodium or dipotassium salt of EDTA (2 5 mmol) was dissolved in approximately 5 ml of water by warming To this $VOSO_4\,5H_2O$ (0 42 g, 1 66 mmol) was added with constant stirring The reaction mixture was stirred for approximately 5 min in an ice-bath Alkali hydroxide pellets were added to this solution to raise the pH to 7 Solid $A[VO(O_2)_2(H_2O)]$ complex (2 5 mmol) was then added in one portion to the reaction mixture with constant stirring The pH of the solution was ultimately raised to 8 5 by the further addition of AOH The initial blue colour of the solution changed to green on addition of yellow ADPV and ultimately a deep red coloured clear solution was obtained within approximately 4 min On addition of pre-cooled C_3H_6O in portions (approximately 5 ml) to the reaction solution under vigorous stirring, a red coloured pasty mass separated The supernatant liquid was decanted off, and the oily residue was treated repeatedly with C_3H_6O under scratching until it became a microcrystalline solid The product was separated by centrifugation, washed with EtOH and dried in vacuo over conc H_2SO_4

Anal Calc for $Na_4[V_2O_3(O_2)_3(EDTA)(SO_4)(H_2O)]\,2H_2O$ C, 16 85, H, 2 52, N, 3 93, Na, 12 92, O_2^{2-}, 4 49, SO_4^{2-}, 13 48, V, 14 32 Found C, 16 78, H, 2 58, N, 3 87, Na, 13 11, O_2^{2-}, 4 44, SO_4^{2-}, 13 41, V, 14 27% Yield approximately 55%

Anal Calc for $K_4[V_2O_3\,(O_2)_3(EDTA)(SO_4)(H_2O)]\,2H_2O$ C, 15 46, H, 2 31, K, 20 10, N, 3 60, O_2^{2-}, 4 12, SO_4^{2-}, 12 37, V, 13 14 Found C, 15 41 H 2 35, K, 20 13, N, 3 62, O_2^{2-}, 3 98, SO_4^{2-}, 12 30, V, 13 16% Yield approximately 30%

The complexes in the solid state were found to be stable for several weeks stored dry at a temperature < 20 °C but tended to be hygroscopic and decompose in a few days at a temperature ≥ 30 °C The compounds were soluble in water

2 3 Elemental analysis

The compounds were analysed for C, H, N, Na and K at the Regional Sophisticated Instruments Centre, North-Eastern Hill University, Shillong, India Vanadium and peroxide were estimated by methods mentioned in earlier papers [16,17]

2 4 Physical and spectroscopic measurements

Spectra in the UV–Vis region were recorded on a Hitachi model 2001 recording spectrophotometer in 1 cm quartz cuvettes The absorbance values are denoted as, e g A_{592}, A_{340}, at the wavelengths indicated The infrared (IR) spectra were recorded with samples as KBr pellets in a Nicolet model 410 FTIR spectrophotometer and also in a Perkin–Elmer Model 983 spectrophotometer The ^1H NMR spectra were recorded in deuterium oxide using a Varian EM-390 90 MHz spectrophotometer Sodium salt of 3-(trimethylsilyl)-1-propane sulphonic acid was used as the internal standard , Magnetic susceptibilities were measured by the Gouy Method, using $Hg[Co(NCS)_4]$ as the calibrant

2 5 Measurement of catalase-dependent oxygen release

A Gilson 5/6 H oxygraph fitted with a Clark oxygen electrode was used for measuring changes in dissolved oxygen (0 224 mM at 30 °C) in the medium and the changes were recorded in units of μM of dissolved oxygen The rate and total amount of oxygen released from a 0 2 mM solution of the compound in phosphate

buffer (50 mM, pH 7 0) on adding catalase (0 08 mg protein ml^{-1}) were measured The recorder pen was set in the middle of the chart paper for measuring the release of oxygen into the medium The machine was standardised by the increases in dissolved oxygen obtained on adding catalase to buffered solutions containing known amounts of H_2O_2

The effect of catalase on complexes was also studied by estimating the peroxide content of the compound **1** at different time intervals in a solution containing catalase The reaction solution contained phosphate buffer (50 mM, pH 7 0), catalase (20 mg) and the compound **1** (50 mg) The volume of the reaction solution was kept at 25 ml The solution was incubated at 30 °C Aliquots of 5 ml were pipetted out and titrated for peroxide content at time 5, 10 20, 30 and 40 min from starting the reaction

2 6 *Measurement of bromination activity*

The method of de Boer et al [18] of introducing four bromine atoms into the molecule of C_6H_5OH red ($\varepsilon^{433} = 19\ 7$ mm) to form the product, bromophenol blue ($\varepsilon^{592} = 67\ 4$ mm) 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 (2 M) and C_6H_5OH red (20 μM) The redox activity was tested by adding the solid compounds 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 in experiments where weighed amounts of solid peroxovanadate samples were added Aliquots were transferred to the spectrophotometer immediately after mixing

2 7 *Measurement of oxidation of NADH*

The absorbance at 340–380 nm by high concentrations of peroxovanadates had to be balanced by adding equivalent amounts in the blank and experimental cuvettes Weighed samples of compound (20 mg/10 ml) were added to phosphate buffer (50 mM, pH 7 0) Only the experimental sample contained NADH (0 2 mM) Immediately after mixing, the sample was transferred to a cuvette and A_{340} was noted No effect was observed on A_{340}

3. Results and discussion

Based on their detailed investigations on V^{IV}–DPV reactions [4–8] and taking into account the redox chemistry of vanadyl, vanadate and peroxovanadates described earlier by Brooks and Sicilio [10] and Jaswal and Tracey [19], it was proposed by Ramasarma and co-workers that a short lived [$OVOOV(O_2)$] species is the intermediate shared by the processes oxidation of NADH [4], formation of oxidised bromine species [5], inactivation of glucose oxidase [6] and release of oxygen [7] Evidence was shown earlier for the participation of the peroxo-bridged divanadate compound in oxidising bromide ion at physiological pH [5,8,9] Observing the inhibitory effect of EDTA and other organic ligands on each of the above-mentioned redox processes [4–7], we realised the distinct possibility of stabilising and isolating the proposed peroxo-bridged intermediate through complexation Using this as a synthetic strategy, the reactions of alkali diperoxovanadate, EDTA and $VOSO_4$ were carried out at varying pH, ranging from 7 to 9 The success of obtaining the solid dinuclear heteroligand peroxovanadates containing SO_4^{2-} and EDTA as heteroligands, as sodium or potassium salts depended on the following essential components maintenance of the molar ratio of DPV VO^{2+} EDTA at 1 0 75 1, order of addition of the reactants, pH of 8 5, maintenance of required reaction time as well as temperature at ≤ 4 °C A solid product isolated at pH 7–8 was found to be EDTA peroxovanadate, which rendered inconsistent analysis

The elemental analysis data provided crucial information regarding composition of the compounds A ratio of 2 1 was ascertained for V peroxide, V EDTA, as well as for V SO_4^{2-} This suggested a dimeric nature of the complex species The elemental analysis results and the molar conductance values obtained from measurement at ambient temperatures (510–522 Ω^{-1} cm^2 mol^{-1}) were in complete agreement with the formulation of the complexes as $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)]\ 2H_2O$ (A = Na or K)

The electronic spectra of the complexes **1** and **2** displayed a broad band at 390–400 nm ($\varepsilon \approx 600$ mm) which has been assigned to the peroxo (LMCT) transition According to previous studies [7], the absorbance at 780 nm of a blue coloured aqueous solution of $VOSO_4$ decreases progressively on adding batches of DPV to the solution The complete absence of the 780 nm band in the spectra of the newly synthesised complexes indicated the oxidative loss of V^{IV} during complex formation Occurrence of vanadium in the complexes **1** and **2** in its + 5 oxidation state was further evident from their being diamagnetic at room temperature and ESR silent The 8-band spectrum (hyperfine splitting **a** = 115 G) characteristic of V^{IV} of an aqueous solution of $VOSO_4$ was not observed in case of the complexes suggesting oxidation of V^{IV} to V^{V} during the course of the reaction

The IR spectra of the complexes **1** and **2** displayed a rich but sufficiently well resolved spectral pattern significant features of which are summarised in Table 1 The bands observed for peroxo groups were in the

range characteristic of a triangularly bonded peroxide [15–17,20] For the binuclear V–O–V unit the antisymmetric and symmetric stretchings were expected in the 700 and 500 cm^{-1} region [20] Accordingly, a medium intensity band observed at approximately 712 cm^{-1} was assigned to a $\nu_{as}(V_2O)$ mode The strong absorption at approximately 950 cm^{-1} was consistent with the presence of a terminally bonded V=O group in the complexes This band was observed to be rather broad in the spectra probably owing to the presence of bridging oxo groups as well as co-ordinated sulphate in the complexes The IR spectra of the complexes displayed a strong broad band with a maximum at approximately 1625 cm^{-1}, typical of co-ordinated carboxylato groups

Table 1
Structurally significant IR bands of $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)]\,2H_2O$ (A = Na or K)

Compound	IR bands (cm^{-1})	Assignment
$Na_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)]\,2H_2O$	3434m	ν(O–H)
	1624s	ν_{as}(COO)
	1404s	ν_s(COO)
	1136s, 1103s	ν(S–O)(ν_3)
	641m, 614m, 464m	ν(S–O)(ν_4)
		ν(S–O)(ν_2)
	945s	ν(V=O)
	712m	$\nu_{as}(V_2O)$
	836m	ν(O–O)
	584s	ν_s(V–O_2)
$K_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)]\,2H_2O$	3420m	ν(O–H)
	1631s	ν_{as}(COO)
	1399s	ν_s(COO)
	1138s, 1105s	ν(S–O)(ν_3)
	638, 612, 468	ν(S–O)(ν_4)
		ν(S–O)(ν_2)
	944	ν(V=O)
	712	$\nu_{as}(V_2O)$
	835	ν(O–O)
	578	ν_s(V–O_2)

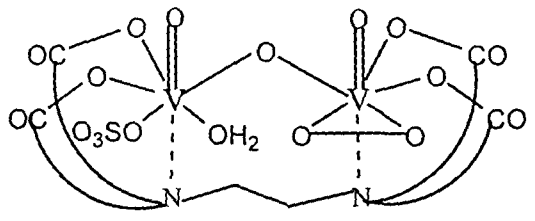

Fig 1 Proposed structure of dinuclear heteroligand peroxovanadate(V) compounds, $A_4[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)]\,2H_2O$ (A = Na, K)

of EDTA [21a] The broadening of the band was possibly caused by the additional OH deformation modes of the water molecules present in the complexes No other band was observed in the vicinity of 1700 cm^{-1} which indicated the absence of free carboxylate groups, thereby suggesting the co-ordination of EDTA as a hexadentate ligand in the complexes The corresponding $\nu_s(COO^-)$ band of EDTA was observed at approximately 1399 cm^{-1} which was shifted from the free ligand value (1412 cm^{-1}) as expected for unidentate carboxylate groups [21a] The presence of water in the complexes was evident from the broad absorption at 3500–3400 cm^{-1}, due to ν(O–H) A band at approximately 755 cm^{-1}, assigned to the rocking mode of water, suggested the occurrence of co-ordinated water The existence of co-ordinated sulphate in the complexes was evident from their IR spectra The well-resolved splitting of the ν_3 and ν_4 modes of SO_4^{2-} into two bands each (Table 1) and appearance of a medium intensity ν_2 mode at approximately 465 cm^{-1} conclusively proved that the sulphate ligand binds the vanadium centre in an unidentate (C_{3v}) fashion [21b] The ν_1 mode of an unidentately bonded SO_4^{2-} expected in the vicinity of 970 cm^{-1} could not be assigned decisively due to its possible mixing with the V=O stretching of terminal oxo groups Significantly, the IR spectral pattern originating from co-ordinated sulphate in the complexes was observed to be very similar to that of vanadyl sulphate, $VO(SO_4)\,5H_2O$ This observation led us to infer that the sulphate–vanadium co-ordination in $VOSO_4$ remained unaltered during its reaction with DPV in the presence of EDTA, being affected neither by the oxidation of V^{IV} to V^V nor by the complexation with EDTA leading to the formation of the newly synthesised complexes

The ^1H NMR spectra of the complexes **1** and **2** exhibited a singlet at δ 2 71 and an AB quartet at δ 3 03 of intensity ratio 2 1 On the basis of previous studies on EDTA complexes, the AB quartet was assigned to the eight acetate protons and the singlet to the four ethylenic protons of the EDTA ligand [22] The close analogy between the NMR spectra of the complexes **1** and **2** and that of a previously reported Mo–EDTA complex [22] containing bridging EDTA suggested the occurrence of the ligand in these complexes as a bridging one, as anticipated by us

Based on the above observations a structure of the type shown in Fig 1 has been envisaged for the complex species An hexadentate EDTA ligand occupying three co-ordination positions around each of the oxo-bridged hepta co-ordinated vanadium(V) centres probably enhances the stability of the dinuclear complexes

To us it appears that EDTA, which is known tc undergo a facile condensation reaction with DPV [11,12] and also forms a stable complex with VO^{2+} binds simultaneously to V^V and V^{IV} centres of th

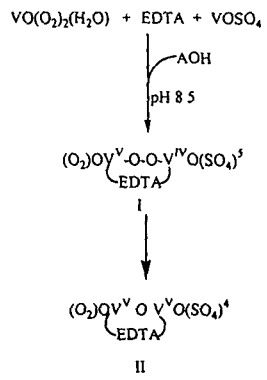

Fig 2 Schematic representation of the formation of the dinuclear heteroligand peroxovanadate(V) complex, $[V_2O_3(O_2)(EDTA)(SO_4)(H_2O)]^{4-}$ from the reaction of diperoxovanadate with $VOSO_4$ in presence of EDTA Hexa co ordination of EDTA is not shown for simplicity No attempt is made to show exact stoichiometry of reactions

μ-peroxovanadate intermediate proposed to be formed in a solution of DPV and vanadyl (Fig 2, complex I) This species then undergoes internal redox involving the reductive cleavage of the bridging peroxo group at the expense of oxidation of V^{IV} to V^{V} leading to formation of the stable dinuclear EDTA and oxo bridged complex (Fig 2, complex II)

The stability of the complexes in solution has been studied by testing the possible oxygen release from a freshly prepared solution of the dinuclear peroxovandate **1** with the help of an oxygraph However, no oxygen was found to be released on dissolution of the complex in water Moreover, the single broad band observed in the 390–400 nm region in the electronic spectra of the complexes showed no change in its position or absorbance over a 2 h period From these observations in combination with molar conductance values the stability of the complexes in water was implicit

Having achieved the synthesis of these compounds we were interested in studying their activity in terms of their ability in bromide and NADH oxidation and action with the enzyme, catalase On incubation with catalase DPV was found to be degraded releasing half the molecular equivalent of oxygen [23] at the rate of 36 0 μM min^{-1} from a solution of 0 2 mM EDTA selectively inhibited this reaction of diperoxovandate with catalase [23] For complexes **1** and **2**, a maximum of 0 5 O_2 per mole of the compound is expected to be released due to the presence of one peroxide group per molecule However, no oxygen release took place from the solution of the complexes **1** and **2** in phosphate buffer (pH 7 0) on treatment with catalase and incubating at 30 °C for up to 30 min, confirming the resistance of the compounds to the enzyme After 30 min, oxygen was found to be released from the solution at an extremely slow rate The exceptional stability of the compounds to resist the catalase action is evidently owing to the extra stability imparted to the complex species by chelation, which probably interferes with the redox changes in the molecule necessary for catalase action

The complexes were unable to bring about NADH oxidation as expected Under the conditions given above, NADH was rapidly oxidised by a mixture of DPV and $VOSO_4$ [4] We now find that addition of the solid compound **1** to solutions of NADH (0 2 mM) failed to oxidise it even at high concentration There was no instant oxidation and A_{340} decreased at a negligible rate (results not shown)

Unlike our earlier experience with peroxo bridged dimeric compounds, $[V_2O_2(O_2)_3L_2]$ (L = amino acid or peptide) which led to the instant bromination of phenol red into its 592-absorbing brominated product, bromophenol blue, at physiological pH [8,9], with the complexes **1** and **2** no such activity was observed

The above observations suggest that the factors such as absence of a μ-peroxo group in the complexes as well as their high stability due to chelation by EDTA are probably responsible for their lack of participation in the above redox processes thus resembling the inhibitor complex formed in solution

In summary, with the examples of the newly synthesised compounds, the present investigation has established that it is possible to isolate the species formed in a solution of diperoxovanadate and VO^{2+} in presence of EDTA which is considered to be responsible for inhibiting the NADH and bromide oxidation and oxygen release reaction by a combination of DPV and VO^{2+} Formation of the complex proceeds through steps where EDTA co-ordinates simultaneously to the V^{V} and V^{IV} centres of the proposed $[(O_2)OV^{V}OOV^{IV}O]^{+}$ intermediate The peroxo bridged species then undergoes internal redox to produce the oxo and EDTA bridged dinuclear peroxovanadate(V) complex (Fig 2, species II), which is resistant to further degradative loss of peroxide and can be isolated in the solid state as its Na^{+} or K^{+} salts It is of interest to note that although SO_4^{2-} is not directly co-ordinated to the peroxovanadate centre, these complexes are probably the only known peroxovanadate compounds containing co-ordinated sulphate The reaction may serve as a paradigm for the synthesis of stable dinuclear heteroligand peroxovanadates if carried out in the presence of suitable organic ligands in lieu of EDTA

A significant finding in the present study is the high stability of the synthesised complexes at physiological pH and their resistance to catalase action This may be relevant in the cellular milieu where H_2O_2 has little chance to survive abundant catalase and glutathione peroxidase By forming peroxo complexes of the above type vanadate may provide a way of preserving cellular H_2O_2 in presence of abundant catalase and make it available for its functions

Acknowledgements

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Peroxo-bridged divanadate as selective bromide oxidant in bromoperoxidation

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Abstract

Diperoxovanadate is effective only in presence of free vanadate in vanadium-dependent bromoperoxidation at physiological pH Peroxide in the form of bridged divanadate complex (VOOV-type), but not the bidentate form as in diperoxovanadate, is proposed to be the oxidant of bromide In order to obtain direct evidence, peroxo-divanadate complexes with glycyl-glycine, glycyl-alanine and glycyl-asparagine as heteroligands were synthesized By elemental analysis and spectral studies they were characterized to be triperoxo-divanadates, $[V_2O_2(O_2)_3(peptide)_3]\ H_2O$, with the two vanadium atoms bridged by a peroxide and a heteroligand The dipeptide seems to stabilize the peroxo-bridge by inter-ligand interaction, possibly hydrogen bonding This is indicated by rapid degradation of these compounds on dissolving in water with partial loss of peroxide accompanied by release of bubbles of oxygen The ^{51}V-NMR spectra of such solutions showed diperoxovanadate and decavanadate (oligomerized from vanadate) as the products Additional oxygen was released on treating these solutions with catalase as expected of residual diperoxovanadate The solid compounds when added to the reaction mixtures showed transient, rapid bromoperoxidation reaction, but not oxidation of NADH or inactivation of glucose oxidase, the other two activities shown by a mixture of diperoxovanadate and vanadyl This demonstration of peroxide-bridged divanadate as a powerful, selective oxidant of bromide, active at physiological pH, should make it a possible candidate of mimic in the action of vanadium in bromoperoxidase proteins (Mol Cell Biochem **236** 95–105, 2002)

Key words bromoperoxidation, bromide oxidant, peroxo-bridged divanadate, diperoxovanadate

Introduction

Vanadium is a native constituent of bromoperoxidase proteins and has an essential role in their catalytic activity [1, 2] Choice of vanadium by this enzyme seems appropriate as its compounds, $VOSO_4$ [3] and V_2O_5 [4, 5], enhanced the extremely slow rate of the chemical reaction of H_2O_2-dependent oxidation and transfer of bromine atom to acceptor molecules Monoperoxovanadate (MPV) and diperoxovanadate (DPV) are readily formed on adding H_2O_2 to vanadate [6–8], with DPV predominating at pH > 5 0 [8] and in phosphate buffer [9] A peroxovanadate, therefore, is likely to be the bromide oxidant Finding such an oxidant of bromide with good activity at physiological pH, an essential requirement of a biomimic model, is the objective of this study

Concentration of DPV, formed from cis-dioxovanadium (OVO^+) and H_2O_2, was found to decrease with concomitant increase in vanadate during bromide oxidation [10] Based on this observation DPV was initially proposed to be the oxidant However, synthetic DPV and MPV could not substitute for $V_2O_5 + H_2O_2$ [11] The proposal was then modified to include a triperoxo complex of divanadate, presumed to form by a combination of MPV and DPV [12], but such a compound was not tested High rates of bromination were

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indeed obtained in these experiments but only at low pH (< 5 0) indicative of reactions and intermediates that suited acid conditions

At pH 5 0 and above, bromination activity is very low with DPV, notwithstanding its possession of both bidentate and linear peroxo groups And the activity obtained with a mixture of H_2O_2 and vanadate is lost when the ratio of H_2O_2 vanadate exceeded 2 1, when all vanadate is converted to DPV [13] Either uncomplexed vanadate (V^V, VO_4^{3-}) or vanadyl (V^{IV}, OV^{2+}) is required for bromination by DPV, and this is substantiated by demonstration of remarkable enhancement rates of bromination on their addition to the system containing DPV alone [13] Ignoring this strong evidence available as early as 1996 proposals continue to implicate the bidentate peroxide as the bromide oxidant Besides bromide oxidation, other reactions also occur when an oxidizable substrate is present during interaction of vanadyl (a reductant) and DPV (an oxidant) These are oxidation of NADH [14], inactivation of glucose oxidase [15], and hydroxylation of benzoate [16] Gaseous oxygen is released in the absence of a substrate

In their classic paper, Brooks and Sicilio [6] showed that vanadyl and H_2O_2 form an unstable addition complex, $OVOOH^+$, which breaks down to OVO^+ and $^{\cdot}OH$ radical Interactions of these reactive products lead to release of dioxygen from residual peroxides Extending this proposal with DPV replacing H_2O_2, we proposed that the corresponding μ-peroxo-bridged-divanadate intermediate, $[OVOOV(O_2)]^{3+}$, is likely to be an active intermediate Importance of μ-peroxo-bridge, present in the protonated form of H_2O_2 (HOOH) in acid pH and retained in the vanadium dimer (VOOV) at higher pH, is recognized in this proposal of bromide oxidation We therefore surmise the active oxidant in naturally occurring vanadium proteins must also be such 'bridged peroxide', to act at physiological pH

Evidence supporting this proposal is available with two synthetic VOOV-type complexes Such complexes of triperoxodivanadate coordinated to glycine $[V_2O_2(O_2)_3(Gly)_2(H_2O)_2]$ [11, 17] and to triglycine $[V_2O_2(O_2)_3(Gly\ Gly\ Gly)_2\ H_2O]$ showed high bromide-oxidizing activity [18] But they have short half-life in water, and their activity was lost rapidly coinciding with parting of the constituents, MPV and DPV [19] To provide insight into actions of protein-bound vanadium needs more information on synthesis, characterization, structure, bonding and reactivity of peroxovanadate complexes with amino acids and peptides (see ref [20] for an overview) Using a set of dinuclear peroxovanadate complexes with glycylpeptides as heteroligands, specially synthesized for this purpose, further evidence is obtained in this study on peroxobridged divanadate acting as a bromide oxidant Ineffectiveness of a representative of these compounds for oxidation of NADH and for inactivation of glucose oxidase, indicated selectivity of VOOV-type intermediate for oxidation of bromide

Materials and methods

Chemicals and solutions

The sources of chemicals are given below ammonium metavanadate and vanadyl sulfate (SD Fine Chemicals, Mumbai, India), hydrogen peroxide (30% v/v), potassium bromide and potassium phosphates (BDH, Mumbai, India), phenol red, EDTA, glucose oxidase (from *A niger*), catalase, and glycylpeptides (Sigma Chemical Co , St Louis, MO, USA) Solutions were made fresh before the experiments in water, doubly distilled in a quartz apparatus after initially passing through milli RO water purification system

Elemental analysis and determination of vanadium and peroxide

The compounds were analyzed for C, H and N at the Regional Sophisticated Instruments Centre, North Eastern Hill University, Shillong, India and at the Department of Organic Chemistry, Indian Institute of Science, Bangalore, India Vanadium was determined volumetrically by titration with potassium permanganate [21], and 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 72 M sulfuric acid (100 ml) and titration with standard cerium (IV) solution [22] The values are given as % by weight of the compounds from which the ratios of V peroxide are derived

Spectroscopic measurements

Spectra in the visible and ultraviolet region were recorded in a Shimadzu double-beam UV 160A or a Hitachi model 2001 recording spectrophotometer in 1-cm quartz cuvettes All the absorbance values are denoted as, e g A592, A340, at the wavelengths indicated The infrared (IR) spectra were recorded with samples as KBr pellets in a Nicolet model Impact 410 FTIR spectrometer The laser-Raman (LR) spectra were recorded on a SPEX Ramalog model 1403 spectrometer The 4880 Å laser line from a Spectra-Physics model 165 argon laser was used as the excitation source The light scattered at 90° was detected with the help of a cooled RCA 31034 photomultiplier tube followed by a photon-count processing system The spectra were recorded at ambient temperatures by making pressed pellets of the compounds The 51V-NMR spectra were recorded in a Brucker AMX 400 FT spectrometer at vanadium frequency 105 190 MHz with the sample 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

Measurement of bromination activity

The method of de Boer *et al* [23] of introducing four bromine atoms into the molecule of phenol red to form the product, bromophenol blue, 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 (2 M) and phenol red (20 μM) kept at 30°C The reaction was started by adding the solid compounds and was monitored by the increase in absorbance at 592 nm of the product formed (ΔA_{592} = 0 0674/μM) In these experiments, the volume of the reaction mixture was kept at 25 ml to enable accurate weighing of small amounts of solid samples of peroxovanadate added After mixing aliquots were immediately transferred to the spectrophotometer to record the jump in A592 This instant change, obtained because the added compound acted directly as bromide oxidant, therefore represents 'instant activity' The steady rate of increase that followed due to residual peroxovanadate is referred as 'secondary rate'

Measurement of catalase-dependent oxygen release

A Gilson 5/6 H oxygraph fitted with a Clark oxygen electrode was used for measuring changes in dissolved oxygen in the medium (0 224 mM at 30°C) and the changes are shown as units of μM of dissolved oxygen The rate and total amount of oxygen released from a 0 2 mM solution of the compound in phosphate buffer (50 mM, pH 7 0) on adding catalase (0 08 mg protein/ml) were measured The recorder pen was set in the middle of the chart paper for measuring the release of oxygen into the medium The machine was standardized by the increases in dissolved oxygen obtained in buffered solutions containing known amounts of H_2O_2 on adding catalase Oxygen release from H_2O_2 was complete within 10 sec under these conditions, but a maximum of only about 200 μM of O_2 can be measured before it comes out in the form of bubbles This indeed limits the concentration of a substrate to 0 2 mM in this method

Measurement of oxidation of NADH

At the high concentrations of peroxovanadates used, their absorbance at 340 nm had to be balanced by adding equivalent amounts in the blank and experimental cuvettes Weighed samples of compound 1 (7–21 mg in 10 ml to give 1–3 mM) were added to phosphate buffer (50 mM, pH 7 0) Only the experimental sample contained NADH (0 2 mM) Immediately after mixing, the sample was transferred to a cuvette and A340 was noted, and its decrease, indicating NADH oxidation, was followed with time

Measurement of glucose oxidase activity

The reaction mixture contained phosphate buffer (50 mM, pH 7 0), glucose (10 mM), and glucose oxidase (4 6 μg protein/ml) and the reaction was started by adding glucose solution The consumption of oxygen was followed in an oxygraph, and the activity was expressed as the rate (μM/min) Pretreatment of glucose oxidase (2 3 mg protein in 10 ml) was carried out in phosphate buffer (50 mM, pH 5 5) by adding weighed samples of (1 5–9 0 mg/10 ml) of solid compound 1 and incubating for 10 min at 30°C A suitable aliquot to give 4 6 μg protein/ml in the reaction mixture in the oxygraph was then tested for the enzyme activity by the rate of oxygen consumption The reagents carried into the glucose oxidase assay medium at this dilution had no effect on the assay

Preparation of diperoxovanadate

Diperoxovanadate (DPV) was prepared by slowly adding equal volume of H_2O_2 solution (40 mM) to a vanadate solution (20 mM) The pH of the mixture was maintained at 7 0 by periodic addition of dilute KOH to avoid formation of decavanadate favored in acid medium [19] From this solution DPV was precipitated by adding 3 vol of cold acetone and dried Analysis of the content of vanadium and peroxide agreed with the formula $K[OV(O_2)_2(H_2O)]$ and a solution of the compound in phosphate buffer (pH 7 0) showed a single major peak in ^{51}V NMR spectrum with a chemical shift at −706 ppm

Synthesis of peroxovanadate complexes with peptides as heteroligands

The ligands of glycine [17] and its tripeptide, glycyl-glycyl-glycine [18] seemed to stabilize the peroxo-bridge and yielded divanadate products Using this strategy, complexes with glycyl-peptides, glycyl-glycine (1), glycyl-alanine (2) and glycyl-asparagine (3) were prepared in microcrystalline form having shades of orange color The common procedure for the synthesis of peroxovanadate complexes of peptides consisted of adding H_2O_2 (30% solution, 15 ml, 132 3 mmol) gradually with continuous stirring to a mixture of solids of V_2O_5 (0 25 g, 1 37 mmol) and the peptides with a molar ratio of V ligand of 2 3 The mixtures were cooled in an ice-bath and kept stirred for about 15 min by which time the solids dissolved yielding red-colored solutions These solutions were all acidic and their pH was recorded to be 2 0 or below No attempt was made to adjust pH in these experiments On adding pre-cooled ethanol (about 50 ml) to these mixtures under continuous stirring, an orange-colored pasty mass separated at this stage After standing for about 15 min in the ice-

bath, the supernatant liquid was decanted, and the residue was treated repeatedly with acetone ethanol (3 1, v/v) mixture under scratching until it became micro-crystalline solid The product was separated by centrifugation, washed with cold ethanol, and dried *in vacuo* over concentrated sulfuric acid The yields were in the range of 32–50% on weight basis These complexes in solid state were found to be stable for several weeks stored dry at < 20°C but tended to be hygroscopic at ambient conditions and decompose in few days (especially compound 2, unavailable for some analyses)

Results

Synthesis and characterization of peroxo-bridged divanadate complexes

The success of synthesis of peroxo-bridged divanadate complexes depended on use of peptides as ligands in acidic medium The procedure included the following essential components avoid the counter ions, retain the acid pH of the mixture as obtained by not using alkali, and limit water to that contributed by 30% H_2O_2 solution added The glycyl-peptides with hydrophobic amino acids, valine and leucine, failed to give a solid product under similar conditions This suggests that interaction of the polar side-chains may have a role in stabilizing the products

The data on elemental analysis gave crucial information on the composition of these compounds A ratio of 2 3 was obtained for both V peroxide and V ligand (Table 1) Apparently neutral with no charge, the compounds can be fitted with a common structure of $V_2O_2(O_2)_3(peptide)_3\ H_2O$ The calculated molecular weights are given in Table 1 Bridging the two vanadium atoms with a peroxide group and a ligand appeared a good way of stabilizing these compounds

The IR spectra of the compounds 1–3 gave clear indication of the presence of coordinated peroxide, coordinated peptide and terminally bonded V = O groups in each of them (Table 2) A bidentate peroxo group bonded terminally to the V(V) center, as in DPV, exhibits a strong ν(O-O) band at 870 cm^{-1} region [24] Appearance of the two ν(O-O) bands in all the complexes, one at c 835 cm^{-1} and another at a lower frequency range of 805–810 cm^{-1} with some broadening, indicated the presence of two structurally different peroxo-groups, the terminal chelated and the bridging types Similar observations were made earlier in the IR spectra of peroxo-vanadate complexes possessing a μ-peroxo group in addition to terminal peroxide [17, 18] The bands at c 540 and c 620 cm^{-1} have been assigned to ν_2 and ν_3 modes of V-O_2 vibrations The spectra enabled clear identification of ν(V = O) at 930–958 cm^{-1} region arising from terminally bonded V = O group [24]

The LR spectra of the complexes complimented their IR spectra They exhibited signals at c 830 and c 805 [ν(O-O) terminal and bridging peroxide], c 630 and c 550 [V-O2 ν_2 and ν_3) coordinated peroxide], and c 930 cm^{-1} [ν(V = O)] The bridging and terminally bonded bidentate peroxo groups can generally be distinguished on comparing their IR and LR spectra since the ν(O-O) vibration for a bridging peroxide, with only a weak dipole, shows a very weak band in IR but appears strongly in LR [25] The band at c 805 cm^{-1} assigned to ν(O-O) was of weak intensity in IR appears strongly in LR This significant observation confirms the presence of bridged-peroxo group in compounds 1–3 (Table 3)

Two distinct bands were observed for the three compounds in the range of 1660–1680 cm^{-1} and 1590–1630 cm^{-1} representing the ν(C = O) (amide) and ν_{as}(COO) of coordinated peptide ligands [25–27] There was no appreciable change in the position of ν(C = O) band in the complexes compared to free ligands indicating that the amide group was not involved in co-ordination In the spectra of the free ligands the symmetric vibration of the carboxyl group occur in the 1400–1410 cm^{-1} region In case of the complexes a medium intensity broad band observed in the range of 1280–1350 cm^{-1} was assigned to ν_s(COO) of bidentate carboxylate group ($\nu_{as} - \nu_s \approx 300$ cm^{-1}) [25] Its mixing with the C-N stretching of amide group expected to occur in this region [27] probably caused the broadening of the band The spectra of the complexes also displayed

Table 1 Elemental analysis of the synthesized peroxovanadate complexes

No compound	Mol wt	% Found in analysis (% calculated from the formula)					Moles/2V atoms	
		C	N	H	V	peroxide	peroxide	ligand
1 $V_2O_2(O_2)_3$(gly-gly)$_3$ H_2O	644	22 80 (22 26)	13 15 (12 98)	4 29 (4 17)	15 90 (15 77)	15 00 (14 84)	2 83	3 09
2 $V_2O_2(O_2)_3$(gly ala)$_3$ H_2O	686	26 35 (26 24)	12 30 (12 24)	4 40 (4 37)	14 60 (14 84)	14 80 (13 99)	3 04	3 07
3 $V_2O_2(O_2)_3$(gly-asn)$_3$ H_2O	815	27 10 (26 50)	14 80 (15 46)	4 20 (4 05)	11 67 (12 52)	12 20 (11 78)	3 14	3 21

Table 2 Data on infrared, Raman and ultraviolet spectra of the peroxovanadate complexes

No compound	Infrared (IR) and Raman (LR) bands, cm^{-1}				UV peak	
	$\nu_s(V\text{-}O_2)$	$\nu_{as}(V\text{-}O_2)$	$\nu(O\text{-}O)$	$\nu(V{=}O)$	nm	A (1 mM)
1 $V_2O_2(O_2)_3(gly\text{-}gly)_3\ H_2O$	(IR) 561 m	613 m	835 s 803 w	958 s	326	0 35
	(LR) 550	595	835 805	955		
2 $V_2O_2(O_2)_3(gly\text{-}ala)_3\ H_2O$	(IR) 572 m	620 m	835 m 803w	949 s	310	0 72
	(LR) 560	630	840 810	960		
3 $V_2O_2(O_2)_3(gly\text{-}asn)_3\ H_2O$	(IR) 578 m (LR) 580	642 m 630	815 m 820 805	930 s 942	322	0 40
DPV	(IR) 522 m (LR) 528	602 s 590	872 s 870	935 s 950	325	0 60

another distinct band at 1366–1405 cm^{-1} range attributable to a bridging carboxylate group ($\nu_{as} - \nu_s \approx 200\ cm^{-1}$) [25] The N-H stretching bands were observed in the 3300–3100 cm^{-1} region as expected from $^+NH_3$ group Other characteristics indicated that the N-H deformation modes (1520–1600 cm^{-1}) remained unaltered in their position and pattern compared to free ligand and that H_2O molecules were present as part of the lattice without coordination to vanadium (V) centers

Based on these observations it may be inferred that the dipeptide ligands, occurring as zwitterions in the complexes, coordinate to the vanadium (V) through carboxylate group. Hydrogen bonding between the side chains might stabilize the molecule in the solid state The structure of these compounds must incorporate the features of V peroxide ligand = 2 3 3, a μ-peroxo-bridge, two bidentate peroxides, two V = O groups, and carboxylate coordination of the ligands with V atoms The proposed structure that includes unidentate carboxylate coordination of two ligands with the two V atoms, bridged by the third peptide through carboxylate-oxygen atoms, and applicable for the three complexes, is shown schematically in Fig 1

Release of oxygen from the peroxovanadium compounds

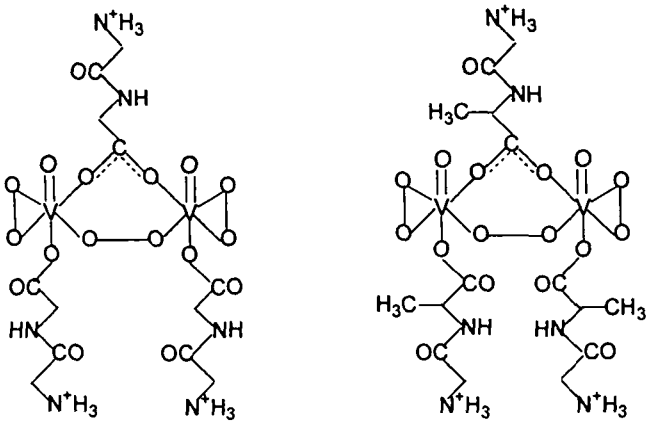
On adding solids of these compounds to water (about 2 mg/ml), bubbles of gas came out of solutions for a few min Oxygen was confirmed to be the gas released at exceedingly high rates of about 50 μM/min for short periods by which time the bubbles formed interfered with the measurement in the oxygraph Instability of the complexes in water and degradative loss of the peroxide groups were implicit This is consistent with the proposal of hydrogen bonds between the amide groups of the peptide ligands providing stability to the complexes

The electronic spectra of the solutions of these compounds recorded after bubbles ceased (about 30 min) displayed a single broad LMCT due to π_ω*-d σ transition originating from coordinated peroxide [17], at 310–330 nm (Table 2) The major product, DPV is known to have a peak at 325 nm [28]

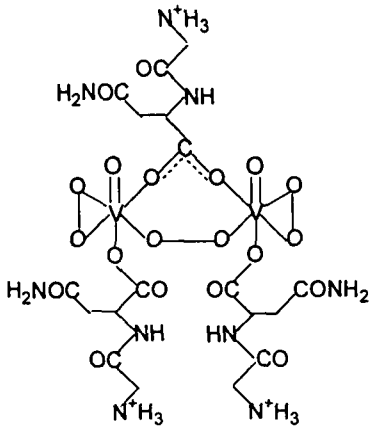
Slow release of oxygen on addition of catalase, indicated that the products in such solutions was peroxovanadate, and is expected to be DPV at pH 7 0 used (Fig 2) Under these conditions reaction with H_2O_2 will be completed in less than

Table 3 Catalase dependent oxygen release from the perroxovanadate complexes

No compound	Conc mM	Oxygen release		ΔO_2/compound (mol ratio)
		μM/min	Total, μM (ΔO_2)	
1 $V_2O_2(O_2)_3(gly\text{-}gly)_3\ H_2O$	0 2	12 3	134	0 67
3 $V_2O_2(O_2)_3(gly\text{-}asn)_3\ H_2O$	0 2	5 0	46	0 23
DPV	0 2	7 0	96	0 48



(1) (2)



(3)

Fig 1 A representation of proposed structures of peroxovanadate-peptide complexes (1) $[V_2O_2(O_2)_3(gly\text{-}gly)_3].H_2O$, (2) $[V_2O_2(O_2)_3(gly\text{-}ala)_3]\ H_2O$; (3) $[V_2O_2(O_2)_3(gly\text{-}asn)_3]\ H_2O$ The ratio of 2 3·3 for V peroxide peptide is accommodated, with one each of peroxide and peptide groups bridging the two V-atoms, and a bidentate peroxide and carbonyl-oxygen coordinated peptide on each of the V-atoms

2 min. This slow release of oxygen from the peroxo groups by catalase is typical of DPV, and is dependent on concentrations of catalase and DPV [28]. The subsequent secondary rates of oxygen release, calculated from these data, paralleled the residual peroxide concentrations. Experimentally values of 0.2–0.7 O_2 per mole of the compound were realized since part of the peroxide was initially lost in releasing oxygen bubbles when making their solutions in water (Table 3). Addition of EDTA (1 mM) decreased the rates to about 15% indicating resistance of their EDTA-complexes to catalase action.

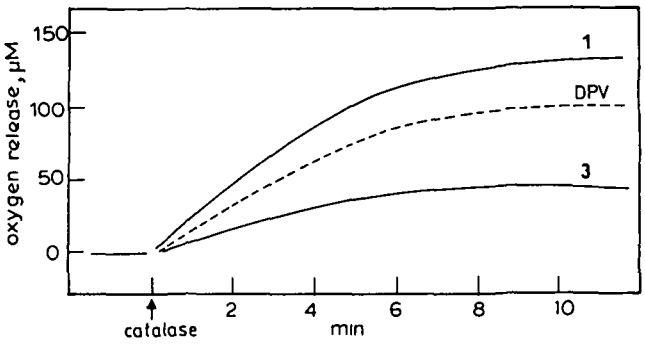


Fig 2 Catalase-dependent oxygen release Solutions of the peroxovanadate-peptide complexes were obtained by adding the solids to water and waiting until the bubbles ceased Aliquots of these were then added to the reaction vessel in the oxygraph, containing the buffer and catalase (0 08 mg protein/ml) The rate and extent of oxygen release were measured (a) gly gly complex, (b) gly.asn complex, DPV (broken line)

NMR spectral analysis of aqueous solution of compound 1 and 3

Using 51V-NMR spectroscopy and assigning the peaks found based on the data available [7, 29–31], the presence of diperoxovanadates in solutions of compounds 1 and 3 was confirmed (Fig. 3). The three peaks, a–c (–426, –509, and –527 ppm with a ratio of 1:2:2), correspond to V_{10}. Traces of residual V_1 (peak d, –545 ppm) and MPV, possibly retaining the peptide ligand, (peak e –650 ppm [30]) are also seen. The major peak f (–695 ppm), is identified with DPV. Small variations from the reported chemical shifts in some cases are likely due to the ligands being still coordinated to some of the products and to variation in pH. Free vanadate (V_1), formed on depletion of its peroxide, is known to oligomerize to decameric form (V_{10}) in acidic solution. The larger proportion of V_{10} in solutions of compound 3 indicates greater degradation, confirmed by its low activity in other systems described below.

Bromination reaction with solid peroxovanadium compounds

The bromination activity of the compounds 1–3 was tested by adding weighed amount of the solid to the reaction medium to study the reaction for the brief period before the molecule broke up. Referred as 'instant' activity, this can be visualized by the solution turning blue immediately after adding the solid and quantitated by the jump in A_{592} (Fig. 4a). After the initial burst of bromination, these reaction mixtures contained DPV and some free vanadate. As expected, the two compounds together gave a secondary rate of bromination

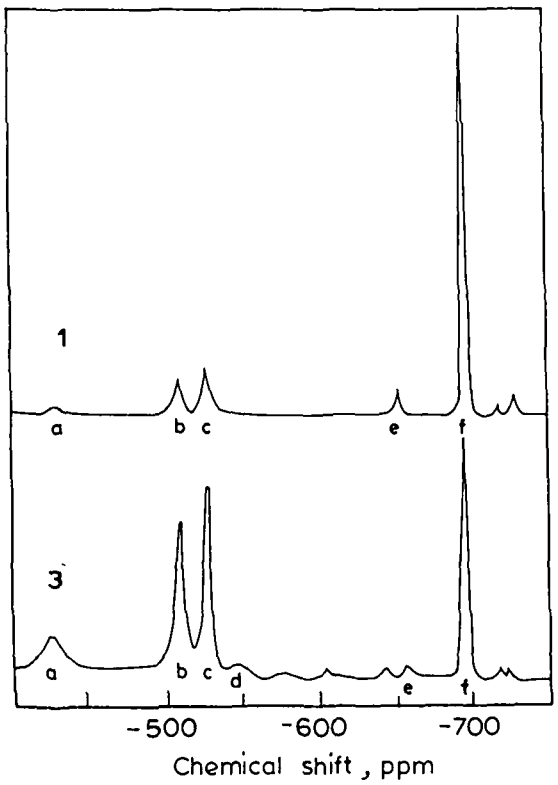


Fig 3. ^{51}V-NMR spectra of aqueous solutions of peroxovanadate-peptide complexes. Solutions of the compounds were obtained as described under Fig. 2. Identification of the peaks. a, b and c, the three peaks (2 2.1) of decavanadate (V_{10}); d, free vanadate (V_1); e, liganded monoperoxovanadate(MPV), f, diperoxovanaate (DPV)

seen as progressive increase in A_{592} indicating increase in the amount of the product (Fig. 4b). Dependable weighments (35–75 mg) of the solids and a large volume of the reaction mixture (25 ml) were used to obtain accurate, comparable data. The data in Table 4 show that all the compounds have the instant and the secondary activities. Considering the extreme instability of these compounds in water, even the small activity only 6–13% substantiated the oxidant capacity of the original compounds. Under these conditions, DPV (1 mM) was completely inactive alone and in the presence of vanadate (0.1 mM) showed the expected secondary rate but not the instant activity (Table 4). Instant bromination activity was realized with these compounds in phosphate buffer at pH 7.0. The data in Table 4 were obtained at pH 5.5 in order to record the secondary activity.

NADH is not oxidized and glucose oxidase is not inactivated by compound 1

Under the conditions given above, a mixture of DPV and $VOSO_4$ rapidly oxidizes NADH [14] and inactivates glucose

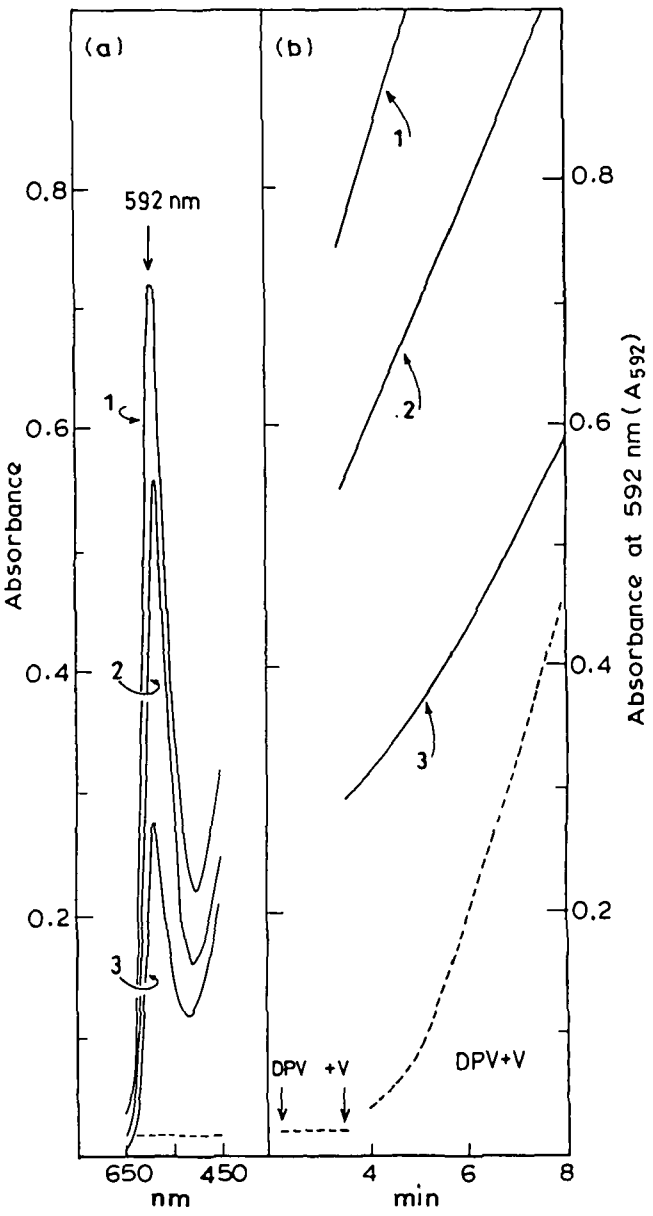


Fig 4 Bromination activity with peroxovanadate-peptide complexes. (a) The spectra were taken immediately after adding the solid compounds to the reaction mixture showing the 'instant activity', (b) increases in A_{592} indicating the secondary rate of bromination by the residual peroxovanadates. The numbers of 1–3 and DPV ($+V_1$) on the lines identify the additions

oxidase [15]. Compound 1 was chosen as a representative for testing these effects.

We now find negligible decrease of A_{340} of NADH on addition of the solid compound 1 to give concentrations of 0.37 and 0.86 mM to solutions of NADH. There was no instant high oxidation as observed when a solution of $VOSO_4$ (0.1 mM) was added to a mixture containing NADH and DPV (0.1 mM) (Fig. 5).

Table 4 Bromination reactions with peroxovanadate complexes

No compound	Conc mg/ml	mM	Instant activity ΔA_{592}	Instant activity bromine transfer total, μM	Instant activity bromine transfer μM/mM compd	Secondary rate (extrapolated to 1 mM compd) μM Br /min
1 $V_2O_2(O_2)_3(gly\text{-}gly)_3 H_2O$	0 30	0 46	0 77	46	100	17 8
2 $V_2O_2(O_2)_2(gly\text{-}ala)_3 H_2O$	0 29	0 42	0 56	33	79	6 6
3 $V_2O_2(O_2)_2(gly\text{-}asn)_3 H_2O$	0 22	0 27	0 28	17	63	6 5
DPV		1 00	nil	nil	nil	nil
DPV (+ vanadate, 0 1 mM)		1 00	nil	nil	nil	4 2

Addition of DPV (0 4 mM) followed by $VOSO_4$ (0 4 mM) to a solution of glucose oxidase (0 16 mg protein/ml) inactivated the enzyme extensively [15] Individually these reagents had no effect The synthetic compound 1 was added as solid to a solution of the enzyme to give final concentrations of 0 5–1 5 mM and the mixture was preincubated for 10 min An appropriate aliquot of this mixture containing treated glucose oxidase (0 16 mg protein/ml) to the reaction medium in the oxygraph showed nearly the same rate of oxygen consumption as the control (Fig 6) indicating that the enzyme was unaffected by the treatment

Discussion

Given to undergo favorable condensation reactions with H_2O_2 in aqueous solutions, vanadate yields peroxovanadates with increasing ratio of peroxide/V on increasing pH and reagent concentration [22, 29–32] Interactions of peroxovanadate with amino acids and peptides in solution have been studied extensively [29–32] A monoperoxovanadate containing gly-gly has been characterized structurally by X-ray crystallography [32] Oxo-bridged peroxovanadates were obtained on stabilization with citrate [33] and lactate [34] An unusual dinuclear complex with both V-atoms sharing the two peroxide-oxygen atoms was obtained in presence of fluoride, a purely inorganic heteroligand [35] Information on bridged peroxide is however limited owing to difficulties in stabilizing it

Presence of a peroxo-group instead of an oxo-group as the bridge in the synthetic compounds is indeed fortuitous in the present study Such complexes could be isolated in presence of amino acids and peptides as heteroligands from highly

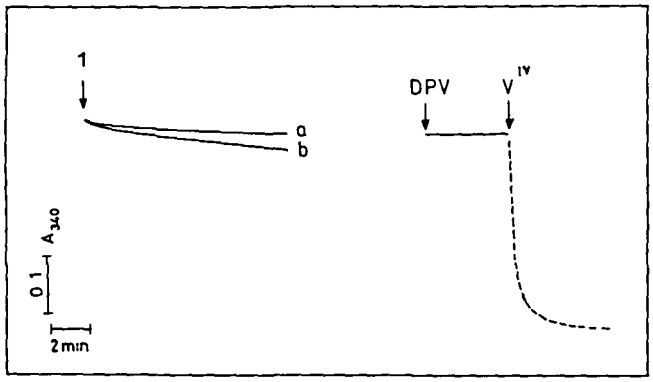


Fig 5 Lack of oxidation NADH by compound 1 Solid compound 1 was added as indicated (a) 0 37 mM, (b) 0 86 mM to a reaction mixture containing phosphate buffer (50 mM, pH 7 0) and NADH (0 2 mM) and the absorbance at 340 nm was recorded A slow rate was found in contrast to rapid decrease obtained with a mixture of DPV (0 1 mM) and vanadyl sulfate (V IV, 0 1 mM (broken line)

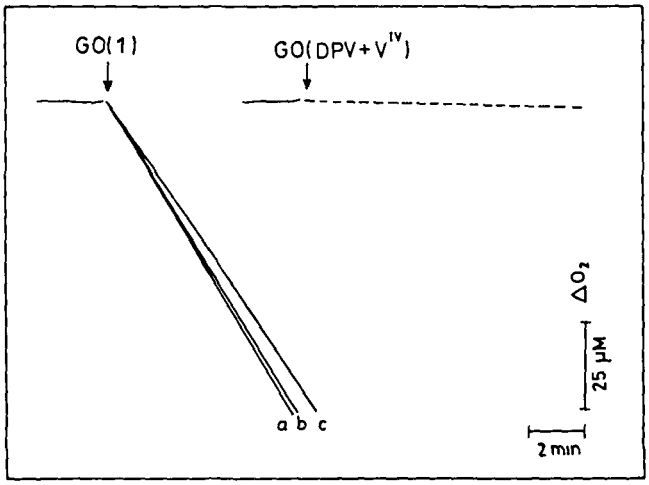


Fig 6 Lack of inactivation of glucose oxidase by compound 1 Solid compound 1 as indicated (a) nil, (b) 0 5 mM, (c) 1 5 mM) or DPV (0 4 mM) followed by vanadyl sulfate (V^{IV}, 0 4 mM) were added to a reaction mixture containing phosphate buffer (50 mM, pH 7 0) and glucose oxidase (1 6 mg protein) and incubated for 10 min at 30°C An aliquot containing 8 mg protein was then transferred to the oxygraph reaction vessel (1 75 ml) containing phosphate buffer (50 mM, pH 7 0) and glucose (10 mM) and oxygen consumption was followed Extensive inactivation occurred only with DPV+ V^{IV} (broken line)

acidic solutions [17, 18] The low pH used allows little deprotonation of amide group and thereby limits coordination of vanadium in these complexes with carboxylate groups of peptide- zwitterions [36] Bridging third carboxylate probably provides additional stability in the solid state It is reasonable to expect enhanced stability of the V-dimer through hydrogen bond interaction with amide groups left free Ready loss of hydrogen bonds in water indeed is the likely cause of the instability of these compounds A similar use of amino acids residues in the bromoperoxidase protein is therefore anticipated

That only VOOV-type intermediate is capable of bromide oxidation at *physiological pH* is obvious from our studies. Any proposal of biomimic of bromoperoxidase can not ignore this Active research groups in this field such as Butler [12], Pecoraro [37] and Conte [38] had been promoting for some time bidentate form of peroxide of vanadium as the active species in halide oxidation Presently accepted mechanism implicates reductive opening of the bidentate-$V(O_2)$ by bromide to form hypobromous species This may be so at acid pH used in their experiments In our experiments at pH 7 0, all the vanadate would be converted to DPV and its peroxogroups certainly could not oxidize bromide Authenticated in two papers [13, 19] in an accessible journal, this awaits noticing Notwithstanding awareness that DPV alone is ineffective, several authors continue to put up 'hydroperoxo complex' of vanadium as the active species for bromide oxidation

A compound, identified as an oxo-bridged triperoxo divanadate by its chemical shift of –670 ppm in ^{51}V-NMR spectrum [29], was found in tiny amounts at high vanadate concentration in highly acidic medium, and was acclaimed to be the 'critical oxidant of bromide' [12] But this offered no special feature of peroxide different from DPV to gain the oxidant activity We could reproduce this experiment but found this NMR-peak remained unaffected in presence of bromide This oxo-bridged complex is, therefore, unlikely to be formed or involved in bromide oxidation under physiological conditions Compounds showing a chemical shift at –760 ppm, suggested to be divanadates, were previously found in the reaction mixture consisting of vanadate, H_2O_2 and glycyl-glycine (pH 6 8) [30] or phenylalanyl-glutamine [39] Some minor peaks in the range of –760 to –770 ppm were in deed found in our experiments in presence of EDTA, and these may represent original, undegraded divanadates

The scheme of reactions shown in Fig 7 is formulated from the work of many laboratories Reactions of the sequence a–e indicate the way oxygen is released from vanadate and H_2O_2 Reaction f recycles vanadyl from vanadate by reduction under acidic conditions and is easily demonstrated experimentally [13] The source of the electron is unclear for this deceptively simple reaction Easy polymerization of vanadate to V_{10} also occurs simultaneously [40] during which packing rearrangement of V-O bonds may provide electrons Essentiality of vanadyl-V^{IV} for making effective oxidants from DPV was accommodated as V^{IV}-OO-V^{V} intermediate in the reaction pathway This represents a mixed valence state

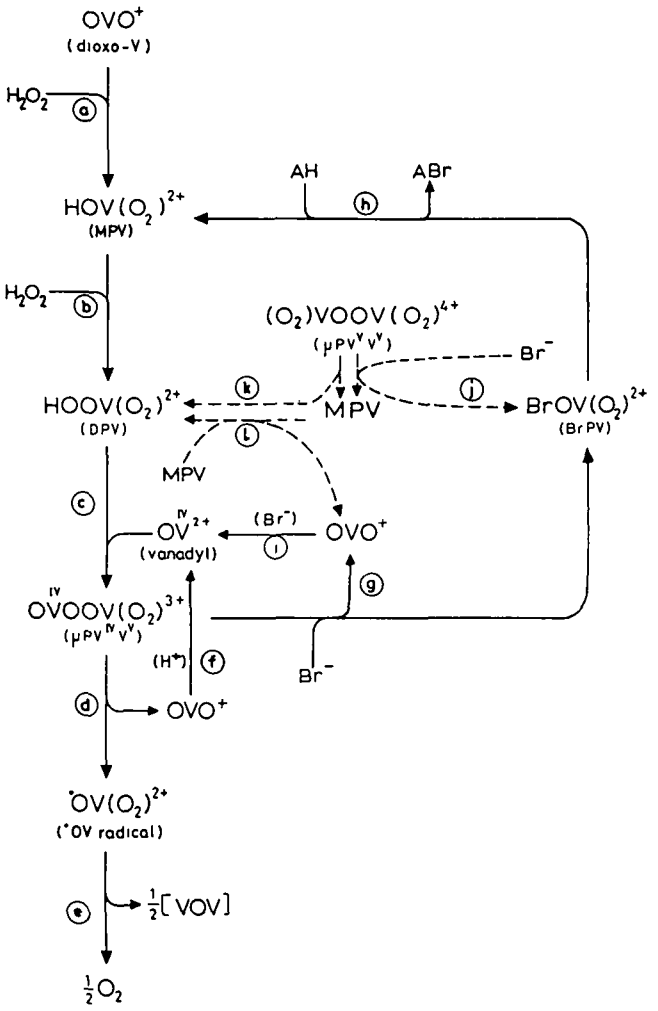


Fig 7 Schematic representation of the peroxovanadate cycle and integration of reactions occurring with peroxo-bridged divanadate compounds (a) formation of monoperoxovanadate (MPV) from vanadate and H_2O_2, (b) formation of diperoxovanadate (DPV) from MPV, (c) formation of a μ-peroxo compound from DPV+vanadyl, (d) breakdown of the μ-peroxo-group, (e) dismutation of OV radical releasing O_2, (f) reduction of vanadate to vanadyl enhanced in acid pH, (g) oxidation of bromide yielding bromoperoxovanadate (BrPV), (h) transfer of bromine atom to acceptor AH (phenol red), (i) bromide-assisted reduction of vanadate to vanadyl, (j) formation of the BrPV when solid divanadate is added to a bromide solution, (k) separation of DPV and MPV on adding solid divanadate compound to water, (l) dismutation of MPV (+MPV) to DPV and vanadate The reaction sequence b-c-g-h-i constitutes peroxovanadate cycle for continuous bromoperoxidation on supplying H_2O_2 Instant activity is obtained by reactions j-h Secondary activity is obtained until MPV exhausts by reactions l-i-c-g-h [VOV] represents a mixture of VO^{3+} and VO_3^+ or a complex thereof as this is non-recycling Ligands in compounds 1–3 are not shown The charges shown are likely to be balanced by phosphate counter-ions Valency state of reduced vanadium is shown as V^{IV}, and all others are V^{V}

having quadrivalent species proposed to possess 'novel capacities of unpaired electrons' [41] Oxygen release is limited to half-equivalent of free vanadate, as the product of reaction e, represented as [VOV], does not recycle In presence of bromide, oxygen release is blocked, and oxidation and transfer of bromine atom to acceptor occur (reactions g and h) with vanadyl being supplied by a bromide-assisted reduction of vanadate (reaction i) Reactions g and i relate to recycling of V^{IV} In experiments with added vanadate in place of vanadyl, we observe c+i+g+h reactions occur together The extra electron in reaction g is therefore likely to be used in reaction i, a one-electron step The operation of the catalytic cycle (b+c+i+g+h), involving a sequence of mono-, di-, μ- and Br-peroxovanadates, was demonstrated for the first time by observing continuous bromination with glucose oxidase reaction supplying H_2O_2 to regenerate DPV from MPV (reaction b) [13]

The novelty in this proposal is conferring the status of a selective oxidant on VOOV-group This is vindicated by the supporting data on synthetic μ-peroxovanadates in the present experiments Reactions with synthetic peroxo-bridged divanadates, shown by broken arrows in Fig 7, are integrated into the cycle For a short period before breakdown, these compounds can oxidize bromide by the 'instant' reaction j followed by its transfer to the acceptor AH (reaction h) Selective activity of the peroxo-bridged divanadate for bromide oxidation is indicated by the inability of synthetic divanadates to support oxidation of NADH and inactivation of glucose oxidation These two systems may therefore be dependent on the next intermediate in the pathway, the 'OV radical Reaction k shows the breakdown of compounds 1–3 when the solids were added to water in the absence of bromide Dismutation of MPV (reaction l) generates DPV and vanadate, which is then reduced to vanadyl (reaction i) This cycle continues to support bromination reaction, in the absence of a source of H_2O_2, until MPV is exhausted

Crystal structures of haloperoxidase proteins from *Curvularis inequalis* [42], *Ascophyllum nodosum* [43] and *Corallina officinalis* [44] are now available Characteristically they have superimposable dimeric subunits with one vanadium atom per subunit Vanadium is bound at the active site in these proteins by a network of highly conserved residues of arginine (two), glycine, serine, lysine and histidine, and also covalently with another histidine, the architecture being similar to evolutionarily-related acid phosphatases [45] There is no clue whether or not the bridged divanadate exists in these enzyme proteins, and is saved in the absence of bromide It is instructive to note that the two vanadium atoms in the subunits are too far apart (at least 40 Å) to make a V-dimer possible [42] In this context it is appropriate to recall the findings of Rehder *et al* [39] that on treatment with excess vanadate, bromoperoxidase purified from *Ascophyllum nidosum* picks up vanadium at a non-specific site in addition to the active site More importantly binding at the second site was enhanced several fold in presence of bromide This becomes even more relevant in conjunction with the ^{17}O-NMR study of Conte *et al* [38] of the enzyme treated with ^{17}O-enriched hydrogen peroxide A broad signal at 593 ppm, indicative of peroxo ligand in symmetric side-on coordination mode, was assigned to the active site monoperoxo- form Also a narrow signal found at 412 ppm as a distinctive feature of this enzyme, was assigned to free, unspecific monoprotonated diperoxo-form $[HVO_2(O_2)_2^{2-}]$ The authors had dismissed this second vanadium as 'an artefact due to partial release of vanadate from the active centre by hydrogen peroxide' But this interests us, as it can serve as the second vanadium essential in our proposal These findings sustain hope that the unspecifically bound diperoxo-V is brought together with monoperoxo-V at the active site to make the active divanadate species

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