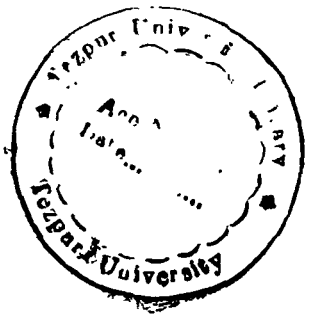

Polymer Anchored Vanadium(V) Compounds: Synthesis and Studies on Their Biochemical and Redox Properties

A thesis submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

by

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Dedicated to -

Hrishi(cousin) and Dikshita(nephew)

ABSTRACT

Polymer Anchored Vanadium(V) Compounds: Synthesis and Studies on Their Biochemical and Redox Properties

ABSTRACT

The present thesis deals with the results of studies involving synthesis, characterization and reactivity of some new vanadium peroxo complexes bound to soluble macromolecular ligands. The thesis also provides an account of the findings of investigation on the effect of the complexes on function of certain enzymes and their antibacterial activity. The contents of the thesis have been compiled into eight chapters.

Chapter 1 presents a general introduction pertaining to the work incorporated in the thesis. The importance of and the interest in vanadium chemistry and biochemistry in general, and peroxovanadium(V) compounds in particular are highlighted. Attention has been drawn to the increasing awareness regarding the utility of water soluble polymers as supports in organic chemistry as well as biology and paucity of information concerning peroxovanadates anchored to soluble polymers. Also emphasized in this chapter is the dearth of reports concerning the effect of heteroligand peroxovanadates and polymer bound peroxovanadates on the activity of different enzymes including phosphatase and the relevance of such studies in the context of contemporary interest involving the potential therapeutic applications of peroxo metal compounds as insulin mimetic agents. This chapter also projects the scope of work on chosen aspects of vanadium chemistry.

Chapter 2 presents the details of the methods of the elemental analyses, and instruments/ equipment used for characterization and structural assessment of the newly synthesized compounds. Methods used for studies on the redox activities of the complexes in bromination and interactions with various enzymes are described herein. The chapter also entails the theoretical background and computational details used for density functional studies on structure and reactivity of a peroxo-bridged divanadate complex.

In **Chapter 3** the synthesis and characterization of a set of new peroxovanadium complexes anchored to water soluble polymers are described. Synthesis of a hitherto unreported monomeric complex with triglycine as a heteroligand is also included herein. Moreover, the results of studies on the thermal stability of the compounds, as well as their stability towards decomposition in solutions of a wide range of pH values are reported in this chapter.

Viable synthetic routes have been developed to obtain the macromolecular peroxovanadate (pV) complexes of the type, $[V_2O_2(O_2)_4(carboxylate)]$-PA [PA = sodium poly(acrylate)] (**PAV**) [**3.1**], $[VO(O_2)_2(carboxylate)]$-PMA [PMA = sodium poly(methacrylate)] (**PMAV**) [**3.2**], $[VO(O_2)_2(sulfonate)]$-PSS [PSS = Poly(sodium 4-styrene sulfonate)] (**PSSV**) [**3.3**], $[V_2O_2(O_2)_4(carboxylate)VO(O_2)_2(sulfonate)]$-P(SS-*co*-M) [P(SS-*co*-M)= poly(sodium styrene sulfonate-*co*-maleate)] (**PSS-co-MV**) [**3.4**]. The methodology was based on the reaction of V_2O_5 with H_2O_2 and respective macromolecular ligand in aqueous medium at near neutral pH. Synthesis of the compounds, in addition to pH, is sensitive to reaction temperature and concentrations of

the components. The pH of the reaction medium was adjusted by adding NaOH. A monomeric anionic peroxovanadate(V) complex containing peptide of the type, $Na[VO(O_2)_2(triglycine)].3H_2O$ (**pV1**) **[3.5]** was isolated from the reaction of V_2O_5 and H_2O_2 and triglycine at pH of 5.5. Each of the above reactions was carried out at an ice-bath temperature ($\leq 4\ ^0C$) and the precipitation of the complexes was brought about by the addition of acetone.

The compounds were characterized by elemental analysis, thermogravimetric studies, SEM, EDX analysis, IR and electronic spectral studies. In **PMAV** and **PSSV**, the pV moieties are anchored in monomeric form to the polymer chain through unidentately co-ordinated O (carboxylate) or O(sulfonate) atoms respectively. Carboxylate groups of PA chain co-ordinate to V(V) centres, in a bridging bidentate fashion leading to the formation of dimeric tetraperoxovanadate structures in **PAV**. In the compound **PSS-*co*-MV** pV moieties are bound to the co-polymer via bridged carboxylate of maleate group as well as unidentate sulfonate groups. The complex $Na[VO(O_2)_2(triglycine)].3H_2O$ contain side-on bound peroxo groups and a triglycine zwitterion binding the metal centre through O(carboxylate) and O(amide) atoms leading to hepta co-ordination around V(V).

Thermal stability of the compounds as well as their stability in solution were determined. Results of peroxide content and position and intensity of their electronic spectral bands confirmed that the compounds are highly stable toward decomposition in solutions of acidic as well as physiological pH.

Chapter 4 deals with the results of investigations on the effect of the enzyme catalase on the polymer anchored peroxovanadates $[V_2O_2(O_2)_4(carboxylate)]$-PA (**3.1**),

$[VO(O_2)_2(carboxylate)]$-PMA (**3.2**), $[VO(O_2)_2(sulfonate)]$-PSS (**3.3**), $[V_2O_2(O_2)_4(carboxylate)VO(O_2)_2(sulfonate)]$-P(SS-*co*-M) (**3.4**) and monomeric heteroligand peroxovandates of the type $Na[VO(O_2)_2(triglycine)].3H_2O$(**3.5**), $Na[VO(O_2)(gly\text{-}gly)(H_2O)].H_2O$ (**4.1**), $Na[VO(O_2)_2(gln)].H_2O$ (**4.2**), $Na[VO(O_2)_2(asn)].H_2O$(**4.3**) and their activity as inhibitors of the enzyme alkaline phosphatase. The reports on antibacterial activity of compounds **3.1-3.4** and **4.1-4.2** are also described in this chapter. Comparisons between the two sets of peroxovanadium compounds viz., monomeric free complexes and polymeric ones could be drawn with respect to their tested properties.

The effect of the above mentioned peroxo-vanadium complexes upon ALP activity of rabbit intestine alkaline phophatase was tested by employing established enzyme assay system and p-NPP (p-nitrophenylphosphate) as substrate. To quantify the inhibitory potential of the molecules, the half-maximal inhibitory concentration (IC_{50}) for each inhibitor which gave rise to a 50% suppression of the original enzyme activity. From the IC_{50} values it is evident that individually each of the tested species inhibited ALP activity with varying degrees. The results show that there is a marked influence of the co-ligand environment on the inhibitory potency of the intact metal complexes. The studies on kinetics of inhibition of ALP demonstrate clearly that mode of inhibition of the free pV complexes on ALP activity is distinctly different from the exerted by the polymer bound complexes. Each of the macromolecular peroxovandium complexes is a non-competitive inhibitor of AL P whereas heteroligand peroxovandates as well as neat diperoxovandate serve as mixed inhibitor of the enzyme.

The effect of catalase on complexes was studied by estimating their peroxide content in a solution containing catalase and phosphate buffer (pH 7.0) at specified time intervals. On incubation with catalase, each of the compounds was found to be degraded gradually with the loss of peroxide. From the rates of degradation of the compounds under the effect of catalase, it was evident that the synthesized peroxovanadium complexes are several fold weaker substrates to catalase as compared to H_2O_2, its natural substrate. The polymer bound complexes displayed greater resistance to the action of catalase compared to the free monomeric pV compounds.

Chapter 5 describes the reports on antibacterial activity of macromolecular peroxovanadium $[V_2O_2(O_2)_4(carboxylate)]$-PA (**3.1**), $[VO(O_2)_2(carboxylate)]$-PMA (**3.2**), $[VO(O_2)_2(sulfonate)]$-PSS (**3.3**), $[V_2O_2(O_2)_4(carboxylate)VO(O_2)_2(sulfonate)]$-P(SS-*co*-M) (**3.4**) and heteroligand peroxovandium $Na[VO(O_2)_2(gln)].H_2O$(**4.2**), $Na[VO(O_2)_2(asn)].H_2O$ (**4.3**) complexes. Attempt has been made to document the comparison between the activity neat diperoxovanadate and polymer immobilized diperoxovanadate.

The inhibitory effect of pV compounds on growth of gram-negative bacterium *E. coli* and gram-positive *S. aureus* was examined by the method of viable counting as well as by monitoring the change in optical density of cultured medium at 620 nm (A_{620}). Experiments demonstrate that each of the tested compounds is effective antibacterial agents against the two bacteria. Marked differences were however noted in magnitude of inhibition of the growth of the two types of bacteria in presence of these compounds. The inhibitory effect of the title compounds both in terms of rate of inhibition as well as MIC values on the growth of *S. aureus* ($p<0.03$) was observed to be significantly greater in

comparison to *E. Coli.* It has been evident that among the tested species the polymer bound pV compounds are the most active antibacterial agents.

Chapter 6 deals with the results of investigations on the reactivity of the soluble polymer supported peroxovanadates complexes, $[V_2O_2(O_2)_4$(carboxylate)]-PA **(3.1)** $[V_2O_2(O_2)_4$(carboxylate)$VO(O_2)_2$(sulfonate)]-P(SS-*co*-M) **(3.4)** in oxidative bromination.

The complexes **PAV (3.1)** and **PSS-*co*-MV (3.4)** efficiently oxidized bromide to a bromination competent intermediate in phosphate buffer at physiological pH. The bromination of phenol red to bromophenol blue was employed to study the bromination activity of the complexes **3.1-3.4** in solution. Addition of freshly prepared aqueous solution of the compound to the standard reaction of bromide in phosphate buffer with phenol red as trap for oxidized bromine resulted in gradual color change of the solution from yellow to blue. The spectrum recorded showed a peak at 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. While the initial addition of H_2O_2 to the reaction solution had no observable effect on the initial rate of bromination, a revival of the bromination activity was noted on addition of H_2O_2 to the spent reaction mixture which contained excess bromide and substrate. The reaction thus could be made catalytic by the addition of exogenous hydrogen peroxide which is apparently required for *in situ* regeneration of the active brominating species. It was of interest to note that the complexes **PMAV** and **PSSV** were inactive in bromination under analogous conditions.

Bromination of several activated aromatics into their corresponding bromo-organics was achieved simply by stirring a solution of the substrate in presence of **PAV**

or **PSSMV** in aqueous-organic media at ambient temperature. The complexes also afforded regeneration and could be reused in fresh cycles of bromination. The observed activity of the polymer bound dimeric tetraperoxovanadate compound **PAV** and **PSS-*co*-MV** in oxidative bromination and inactivity of **PMAV** and **PSSV** is consistent with the proposal implicating formation of a peroxo-bridged divanadate intermediate which is active in bromination.

Chapter 7 presents the results of theoretical investigations on the structure and reactivity of peroxo-bridged divanadate complex, $[V_2O_2(O_2)_3(glycine)_2]$.

It has been reported previously that dinulear peroxovanadium complexes with bridging peroxo group exhibit unique redox properties and could act as powerful bromide oxidant at physiological pH. On the other hand, monomeric diperoxovanadate compounds with exclusively η^2-peroxo groups in its co-ordination sphere were catalytically incompetent in bromide oxidation at neutral pH. The proposed reaction pathway confers the status of a selective bromide oxidant, at physiological pH, on VOOV group. However, the cause of this greater reactivity of a peroxo-bridged divanadate moiety compared to a η^2-peroxo group was not completely clear. In order to gain an insight into this aspect of the complex species containing VOOV, in the present work the structural and electronic properties of peroxo-bridged vanadium complex, $[V_2O_2(O_2)_3(glycine)_2]$ has been investigated using density functional methods. The Fukui function and relative nucleophilicity values were calculated using Mulliken and Hirshfeld population schemes in order to understand the reactivity of O-O moietis.

The results of the study demonstrate that the bridging peroxo and side-on bound peroxo groups present in the complex investigated are nonequivalent structurally as well as in terms of their electrophilicity and hence reactivity It is evident that greater electrophilicity of the O-atoms of VOOV moiety make them more susceptible to nucleophilic attack by the Br in an oxidative bromination process, which presumably leads to the reductive cleavage of the O-O bond with concomitant oxidation of bromide The observations are in harmony with the suggestion that a 'VOOV' group may be the principal requirement for bromide oxidation by peroxovanadate compounds, at neutral 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 major part of the results of studies described in **Chapters 3-6** have been published and rest is under communication

Chapters 3, 4, 5

1 *Synthesis characterization reactivity and antibacterial activity of new peroxovanadium(V) complexes anchored to soluble polymers*
Diganta Kalita, Swapnalee Sarmah, Siva Prasad Das, Ashok Patowary Diganta Baishya, Sashi Baruah Nashreen S Islam*
Reactive and Functional Polymers (Vol 68, 4, 2008, p 876-890)

2 *Kinetics of inhibition of rabbit intestine alkaline phosphatase by heteroligand peroxo complexes of vanadium(V) and tungsten(VI)*
Diganta Kalita, Siva Prasad Das and Nashreen S Islam*
Biological Trace Element Research (accepted)

3 *Peroxovanadium complexes anchored to soluble polymers synthesis antibacterial behaviour and effect on alkaline phosphatase activity*
Diganta Kalita[a], Siva Prasad Das[1] Jeena Jyoti Boruah[a], Alka Kumari[b], Sashi Boruah[b*], Nashreen S Islam[a]*(communicated)

Chapter 6

1. *Density functional study of structure and reactivity of a dinuclear peroxovanadate(V) complex*
   Diganta Kalita, Ramesh Ch Deka* and Nashreen S Islam*
   Inorganic Chemistry Communications Vol 10. 1. 2007 p.45-48

Declaration

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

Diganta Kalita

Date: 12-9-08 (Diganta Kalita)

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I certify that the thesis entitled "***Polymer Anchored Vanadium(V) Compounds: Synthesis and Studies on Their Biochemical and Redox Properties***" submitted by Mr. Diganta Kalita for the degree of Doctor of philosophy of Tezpur University, embodies the record of original investigation carried out by him under my supervision. He has been duly registered, and the thesis presented is worthy of being considered for the Ph.D. Degree. This work has not been submitted for any degree of any other university.

Date: 12.9.08

Place:

N. S. Islam
Signature of the Supervisor

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List of Abbreviations

ALP	alkaline phosphatase
ADPV	alkali diperoxovanadate
AH	acetylene hydratase
DPV	diperoxovanadate
E coli	*Escherichia coli*
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
FDH	formate dehydrogenase
FMDH	n-formylmethanofuran dehydrogenase
HIV	human immunodeficiency virus
HMPA	hexarnethylphosphoric triamide
HPLC	high Performance Liquid Chromatography
Triglycine	glycyl-glycyl-glycine
gly-ala	glycyl-alanine
gly-gly	glycyl-glycine
gly-leu	glycyl- leucine
gln	glutamine
IC_{50}	half-maximal inhibitory concentration
IR	infra red
insRTK	insulin receptor tyrosine kinase
LMCT	ligand to metal charge transfer
MPV	monoperoxovanadate
NAD	nucleotide adenine dinucleotide
NADH	nucleotide adenine dinucleotide reduced form
ndt	2,3- naphthalenedithiolate
NMR	nuclear magnetic resonance

NTA	nitrilotriacetic acid
O-phen	o-phenanthroline
pV	peroxovanadate
PA	sodium polyacrylate
PMA	sodium polymethacrylate
PSS	poly(sodium styrene sulfonate)
PSS-co-M	poly(sodium styrene sulfonate-co-maleate)
pic	picolinato
p-NPP	*p*-nitrophenyl phosphate
p-NP	p-nitrophenol
pV	peroxovanadate
pW	peroxotungstate
PTPase	phosphotyrosine phosphatase
PTC	phase transfer catalyst
RNA	ribonucleic acid
TG	thermogravimetry
tacn	triazacyclononane
DTG	differential thermogravimetry
S. aureus	*staphylococcus aureus*
TMB	1,3,5-trimethoxybenzene
V-BPO	vanadium bromoperoxidase
V-HPO	vanadium haloperoxidase

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CHAPTER 1

1.1 VANADIUM – DISCOVERY AND NATURAL OCCURRENCE

Vanadium, a transition element belonging to Group V, is widely dispersed throughout the earth's crust[1]. First discovered in 1801 by mineralogist A. M. del Rio in a brown lead mineral from Mexico[2,3], vanadium was named as *panchromo* owing to the varied colours of its compounds, but subsequently changed the name to *erythronium* (red) because of the red colour of its salts when treated with acids[2]. Vanadium was rediscovered[4] in the year 1831 by the Swedish chemist Sefström after del Rio had, mistakenly, withdrew his discovery. Impressed by the rainbow of colours of the compounds of the new element discovered, its discoverer named the element as *vanadin* after *Vanadis*, the Norse goddess of beauty. In the same year, Wohler established the identity of erythromium and vanadium[5].

It is the fifth-most abundant element making up about 0.014% of the Earth's crust. It exists naturally in a number of minerals in oxidation states III, IV and V. 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. It is found in relatively high concentrations in certain crude oils and coal deposits where it is present in the form of organic complexes. In ocean it is the second most abundant transition element (50nM)[6]

Vanadium, being ubiquitous in nature1, it is normally present at very low concentrations in virtually all cells in plants and animals[7]. Several ascidians accumulate high concentrations of the element in lower oxidation states in their blood

cells[8]. However, the nature of vanadium species and its role in these bio-systems remain unclear[9]. Vanadium occupies active site metalloenzyme centres and is involved in the activation /inhibition of key metabolic enzymes in biological milieu. Among the metalloenzymes harbouring V in their active sites are the haloperoxidases found in marine organisms[10] and certain nitrogenases of nitrogen-fixing bacteria (*Azotobacter*)[11]. Some accessory foods such as black pepper, tea leaf, cocoa powder and some mushrooms contain relatively high amounts of vanadium.

Much of the driving force for studies of vanadium compounds can be attributed to the recognition of the potential biochemical and therapeutic importance of this element[7,12-29], besides its utility as an oxidation catalyst in industrial processes[30,31]. The vanadium oxide, V_2O_5 is finding recent application in nanomaterials[32].

1.2 BIOLOGICAL SIGNIFICANCE OF VANADIUM

Status of vanadium has been raised to one of high biological relevance from that of a low adventitious contaminant, owing mainly to several major discoveries on biological effects of vanadium over the last two decades[7-11, 33-38], some of which are listed in Table 1.1.

The inhibitory effect of vanadate towards phosphatase was established in 1977 when Cantley and co-workers[39] 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[40,41]. These reports marked the resurgence of interest in finding anti-diabetic vanadium compounds with low toxicity[7,12,18-21,25-27,42-45] and identification of peroxovanadates as possible active compounds that activate directly the cascade of enzymes that normally follows activation of insulin-receptor[46]. 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[47]. 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[14]. Finally, with the discoveries of proteins containing bound vanadium as a native constituent and essential for the activity of a bromoperoxidase[10] in a marine alga, in the year 1983, and of nitrogenase in *Azotobacter*[11] the biological role of vanadium has been firmly established.

The pharmacological value of metavanadate was recognized a century ago in France and it was acclaimed as "*Panacee Universelle*" for treatment of a number of diseases as diverse as anemia, tuberculosis, syphilis and diabetes[37,38,48].

Table . 1.1 *Resurgence of interest in biological actions of vanadium*[38]

Reaction/Parameter	Vanadium	Effect/Locale	Reference
Na,K-ATPase	vanadate	inhibition	39
Insulin-mimic	vanadate	blood glucose	41
Insulin-mimic	vanadyl	blood glucose	40
NADH-O_2 oxidation	polyvanadate	plasma membrane	48
Bromoperoxidase	vanadate	marine alga	12
Nitrogenase mutant	vanadate	*A. vinelandii*	13

. A metavanadate containing tonic (neogadine) is available in the market in India. Most food materials used for human consumption contain vanadium in concentrations[49] below 0.1 μg/g. Dietary supplement of vanadate increases its tissue content which is stored in a non-toxic form[50]. However, pharmacological potential of vanadium has been systematically explored only in the last decade or so[14]. 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[42]. Yet, they have limited clinical usefulness so far due to several factors including toxicity of the metal[51].

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 [6,8] as well as in catalytic oxidations[52-54].

1.3. CO-ORDINATION CHEMISTRY AND BIOCHEMISTRY OF VANADIUM – SELECTED ASPECTS

During the last 15 years an explosive growth has occurred in the chemistry and biochemistry of vanadium and its compounds. Vanadium has a chemical versatility that is useful to biological systems[27,53-61] and is redox active under physiological conditions[54,62-64]. In order to understand how the metal might function in relatively complex biomolecules as well as its role in catalytic oxidations, 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 three highest i.e. +3, +4, and +5 are important in biological systems[65]. Upon dissolution, vanadium in oxidation states III, IV and V can undergo hydrolytic, acid/base, condensation and redox reactions. Vanadium(IV) and vanadium(V) oxidation states are more common and are stable under ordinary conditions[65]. 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[66]. Vanadium studies remained in low profile due to its exceptionally complicated chemistry in solution[67]. However, many of the recent advances in vanadium chemistry derive from the increased recognition that modern spectroscopic tools are highly informative when applied to the study of vanadium compounds.

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[68]. Vanadyl interacts readily with carbonate[22], phosphates[69,70], pyridine, imidazole and other amine bases and form different complexes. Hydrocarboxylic acid, phosphocarboxylate, nucleosides, nucleotides, catecols[69,70] etc. which contain more than one functionality form strong complexes with vanadyl cation. These reactions are

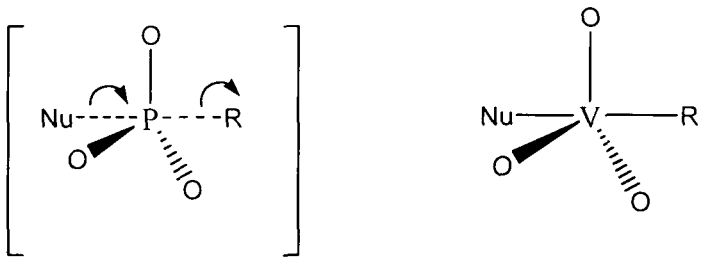
of physiological interest. Interaction of vanadyl with cysteine, cystene[19], picolinic acid[21], N,N-ethylenediamine diacetic acid[71] etc. forms complexes which possess promising insulin-mimetic properties. Bis(maltolato)oxovanadium(IV) (BMOV) is a compound recently developed for oral treatment of diabetes mellitus[7,72].

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[73]. These correspond to OVO^+, VO_4^{3-}, $HVO_4^{2-}(V_1)$ and $H_2VO_4^-(V_1)$ which result from a series of complexes, 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. Vanadium is stereochemically flexible with coordination geometries ranging from tetrahedral and octahedral to trigonal pyramidal and pentagonal bipyramidal being thermodynamically plausible[74]. 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[73,75]. Commonly used organic buffers and EDTA form complexes with vanadium compounds[73].

Vanadate has long been recognized as a structural and electronic analogue of phosphate , encompassing the tetrahedral ground state and the penta coordinated exctited state , which are of chemical basis of the wide range of vanadate biological activities[76]. A large variety of phosphate metabolizing enzymes including phosphatase, mutases, ATPases, kinase, lyases, and some ribonucleases, catalyze phosphoryl transfer reaction, and share same mechanism involving the formation of

penta coordinated phosphate as high energy transition state .In general these enzymes are potently inhibited by vanadate due to the chemical similarities between vanadate and phosphate combined with the ability of vanadate to readily undergo changes in coordination geometry[76-78].



High energy transition state stable state

Fig. 1.1 Schematic representation of penta coordinated phosphate transition state and its vanadate analogy

The vanadium-dependent bromoperoxidase is now the subject attracting much attention of chemist as well as biologist. Studies on synthetic models of V-BPO have been extremely useful in helping to unravel details of the structure and mechanism of activity of the enzyme[55]. Several novel coordination compounds of vanadium have been discovered recently .The advances have been fueled by a symbiotic relationship that is developing between the biochemistry and coordination chemistry of vanadium. The understanding of co-ordination chemistry of vanadium places the new

advancement and directions of biological vanadium into proper perspective. Selected structural models[55] of the enzyme are shown in *Fig.* 1.2.

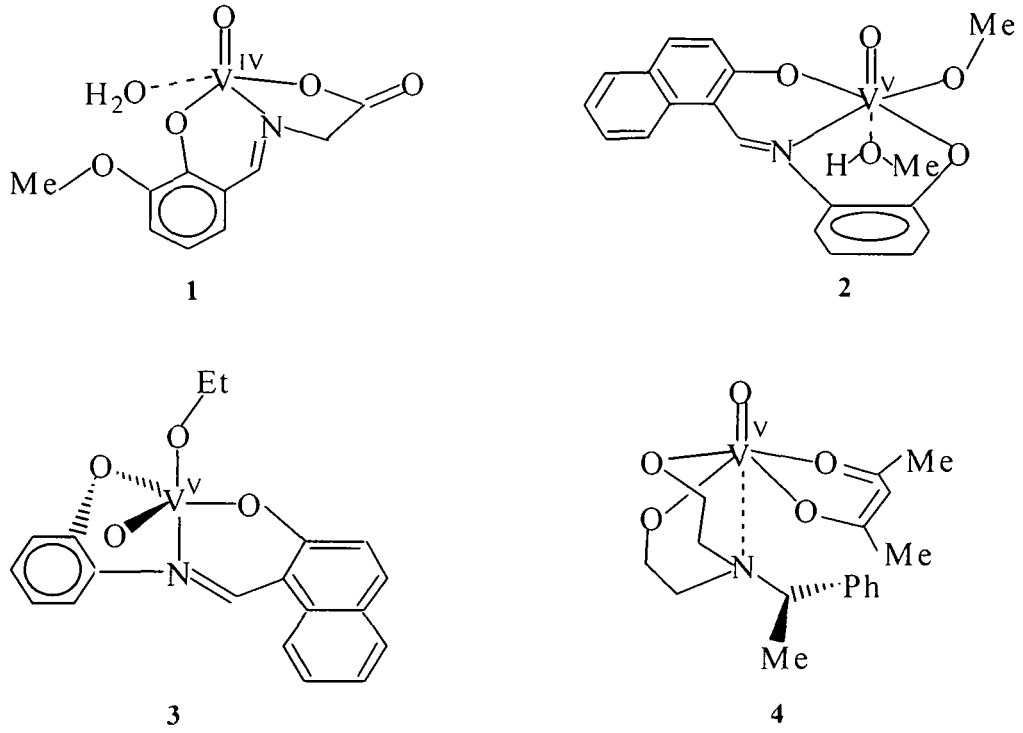


Fig. 1.2 Structural models for the vanadium site in peroxidases[64]. A dashed line (in **1**, **2** and **4**) represents a weak bond. The supporting ligands are Schiff bases (**1-3**) or ethanolamine (**4**)

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[55].

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[27,46,53,79-85]. Peroxo-transition metal complexes in general have received continued attention over several years because of their important roles in biological processes [86-88] and in catalytic oxidations[89-97].

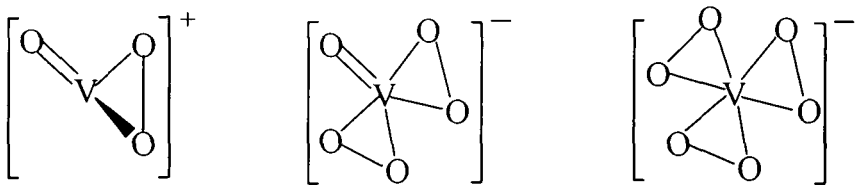
1.4 PEROXO COMPOUNDS OF VANADIUM – CHEMISTRY AND IMPORTANCE

It has been known for over a century that characteristic colour reaction may take place when hydrogen peroxide is added to solutions of transition metal derivatives[98] and many peroxo transition-metal compounds have been isolated in the solid state[53,98-100]. There is currently considerable synthetic interest in the chemistry of peroxometallates[53,84,99]. Besides their scientific significance, such systems are attractive as potential catalysts in biological [43-47,101] and industrial processes or their simple models[84 102-110]. 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[111,112].

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[84,98]. 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[84,98,113,114].

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. At higher peroxide and vanadium ratio and pH > 8.0 triperoxo[115] and tetraperoxo species dominate. Most species have pH dependent ^{51}V-NMR chemical shifts arising from protonation and deprotonation reactions and were characterized by ^{51}V-NMR spectroscopy[84,98.116-118]. 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[84,98,113-115] include the following:

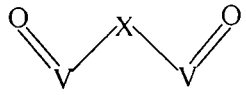
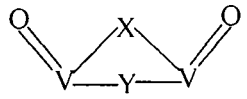
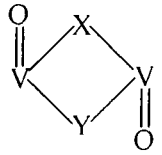
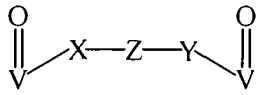
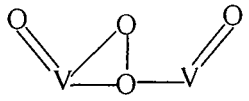
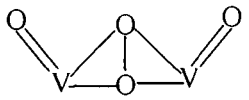
(i) The number of peroxo groups per vanadium atom increases with alkalinity.

(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[12,53,79,87]. Thus, depending on the pH and reaction conditions monoperoxo, diperoxo or triperoxo complexes may be formed which are represented by the formulae : $M_n[VO(O_2)_2(L)]$, $M_n[VO(O_2)_2(LL')]$ or $M_n[VO(O_2)(H_2O)(LL'L'')]$ where M = NH_4, Na or K; n = 1-3; and L, LL' and LL'L'' are mono, bi- and tridentate ancillary ligands[12]. A large number of peroxvanadium and oxodiperoxovanadium(V) complexes in diverse ligand environment have been structurally characterized and reported in recent years[12,53,79,82,119,120]. 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. Some dinuclear peroxovandate 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[82](Table1.2). Examples of structurally characterized dinuclear peroxovanadates are listed in (Table 1.3) . 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[79]. A set of oxo-bridged peroxovanadates synthesized in our laboratory exhibited remarkable

Table 1.2. *Bridge configurations found in dinuclear vanadium(V) peroxo complexes*[82]

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		μ—η^1 : η^2O_2
F		μ—η^2: η^2O_2

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

Table 1.3. *Structurally characterized dinuclear peroxovanadates*[82]

Dinuclear	Bridge	CN	Ligand(s)	Ref.
$[V_2O_2(O_2)_2)L_r]$ r = 4, 5	C,-	7	citrato	53
	B, A	7	L-tartrato, H_2O	82
	B, A	7	D-tartrato, H_2O	82
	C,-	6	glycolato	82
	C,-	6	DL-lactato	82
	C,-	6	DL-mandelato	82
	A, D	7	dpot	53
$[V_2O_2(O_2)_3)L_p]$ p = 3, 4	A, F	7	3F	53
$[V_2O_2(O_2)_4)L]$	E, -	6	H_2O (3)	53
	A, E	6-7	O	53
	A, E	6-7	OH (2)	53

A, B, C, D, E and F denote the type of bridging described in Table 1.2.

resistance to catalase[121,122]. Dinuclear peroxovanadate intermediates possessing a μ-peroxo bridge have been implicated in certain biochemical processes[59,123-126]. However, only a few reports regarding chemistry of such species of vanadium in solid state are available[127-129].

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[113,114,116-118,130], ^{17}O-NMR spectroscopy[131,117], Raman spectroscopy[118] and by electrospray ionization mass spectrometry (ESI-MS)[132-134]. Moreover, structures of vanadium peroxo derivatives are also being theoretically investigated[132,134].

Infrared spectra are essential for the characterization of peroxovanadate compounds[135]. Coordination of peroxide in a side-on bidentate fashion creates a local C_{2v} symmetry which has three IR active modes, symmetric O-O stretching, symmetric metal-peroxo stretching, and antisymmetric metal-peroxo stretching which occur at approximately 880, 600 and 500 cm^{-1} respectively[53]. 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. 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 $\lambda_{max} \sim 320$ nm in diperoxo complexes whereas monoperoxo complexes absorb at a much lower energy with the $\lambda_{max} \sim 420$ nm[79].

The efficiency of peroxovanadium complexes in oxidizing certain organic[84,91-93,136] and inorganic substrates[53,137,138] 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 [53,102,103,132,139], primary and secondary alcohols to the aldehydes and ketones[53,132,140,141], aldehydes to esters[142,143], sulfides to sulfoxides and sulfones[53,93,95], as well as, hydroxylations of alkanes and arenes[53,95,102,144] (*Fig. 1.4*). The catalytic applications of peroxovanadates take advantage of the increased oxidation rate of peroxovanadium complex, which after formation reacts[145]. Owing to the biological significance of the peroxo-vanadate mediated oxidations, most of the recent studies have been conducted in water[128,132,146,147]. However, peroxovanadate compounds are also efficient oxidizing agents in less polar organic solvents[103,148,149]. Recent developments have been utilizing biphasic, phase transfer systems as well[150,151].

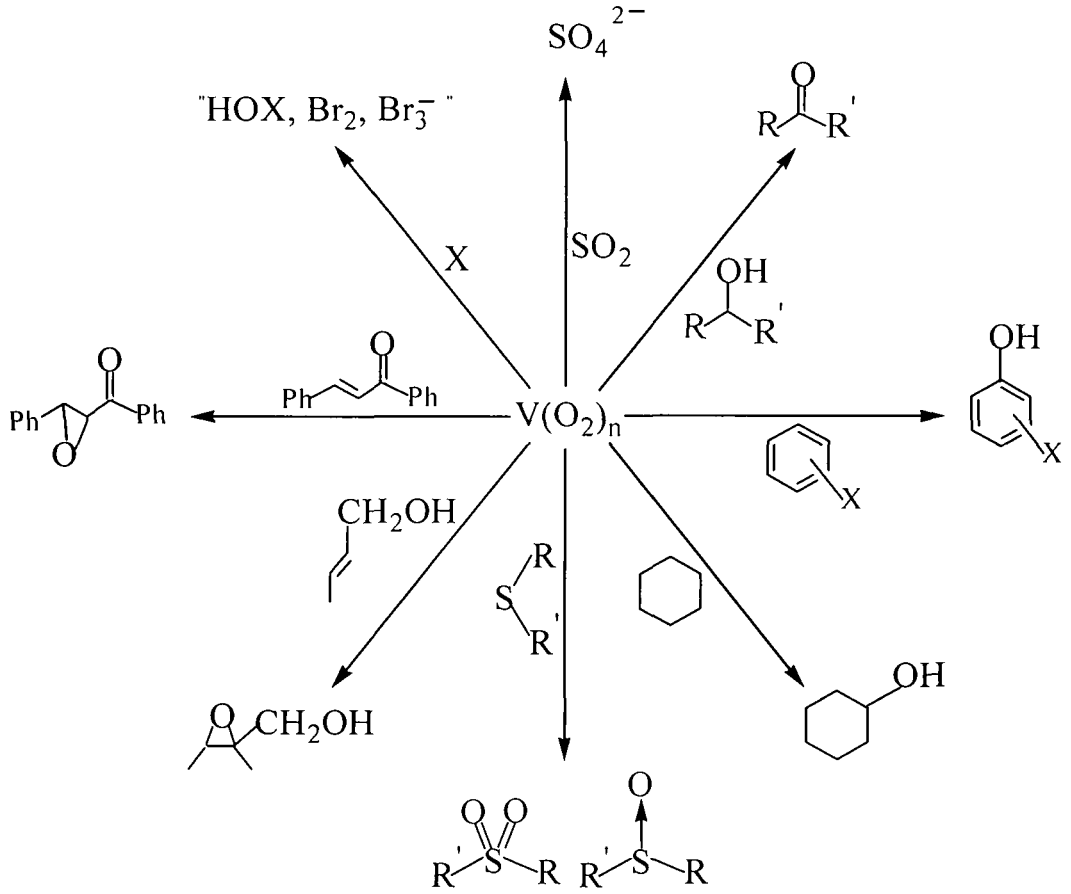


Fig. 1.4 Reactivity of vanadium peroxides with inorganic and organic substrates[132]

The stoichiometric or catalytic oxidations accomplished by peroxovanadates are usually carried out under mild conditions with good selectivity and chemical yields[53]. Mimoun el al. synthesized a series of vanadium complexes of tridentate Schiff base ligand which exhibited clean stereoselective epoxidation[103]. 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[149]. One of the most striking features of the V(V) peroxo

complexes is their ability to oxidize arenes, alkanes and alcohols[53, 102, 132,145-150] Complex $VO(O_2)(OR)$ catalyze the oxidation of 2-propanol to acetone stoichiometrically with respect to H_2O_2 consumption[53,146]. 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[149].

The nature of the coordinating ligand and the solvent system are very important factors on which the oxidative reactivity of peroxovanadate complexes depends[152]. 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[150]. 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[145-147,152,153]. The mechanism of oxidation reactions mediated by peroxovanadates as electrophilic or radical oxidants have been studied extensively[53, 103, 139, 147].

Besides the oxidations of organic substrates, peroxovanadium complexes are also able to oxidize various inorganic substrates including of sulfur dioxide[137], thiocyanate[138] and halides[53,154]. 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[53,59].

The discovery of vanadium bromoperoxidase, the first enzyme found to use vanadium as a cofactor, has resulted in one of the most active areas in vanadium chemistry and biochemistry and also reactivated the interest in vanadium peroxide chemistry. Haloperoxidases are enzymes that catalyse the two-electron oxidation of

halide (chloride, bromide, iodide$^-$) by peroxide to the corresponding halogenating species X_2, X_3^- or hypohalous acid, which can results in halogenations of organic substrates RH [10.33, 53,59. 155]

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

The primary oxidized intermediate is still not known although for bromide it is equivalent of hypobromous acid (HOBr), bromine Br_2), tribromide(Br^{3-}) or an enzyme-bound bromonium ion-type species(e.g. Enz-Br, Venz-OBr,Enz-HOBr,etc). 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 which often have important biological and pharmacological activity[155]. 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[53.156]. 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[53,93, 95,132].

Crystal structures of some haloperoxidase proteins *Curvularis inequalis*,[157] *Ascophyllum nodosum*,[158] *Corallina officinalis* [159]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[160]. The oxygens are hydrogen bonded to several amino acid residues of the

protein chain. Addition of peroxide converts the arrangement from trigonal-bipyramidal to tetragonal pyramidal with the peroxo ligand in the tetragonal plane and oxo-oxygen in the apical position. Quite interestingly, bromoperoxidase show phosphatase activity after removal of vanadate and the peroxidase activity can be restored on reconstitution of the apoenzyme with vanadate.

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[53,55-61,154]. The biomimetic functional models reported in the literature, some of which are discussed in Chapter 6 of this thesis, are mostly based on monoperoxo[56] vanadium or on triperoxo divanadium species[38,59,60,123,124]. Aqueous solution of cis-dioxovanadium(V) (VO_2^+) in acidic medium[60], a V_2O_5 and H_2O_2 system[61] as well as , KBr in excess H_2O_2 in presence vanadyl sulfate in phosphate buffer[156] (pH 6) were all found to be effective in bromination of organic substrates and were studied in detail as functional mimic of the enzyme.

Concomitant with the biochemical interest on the activity of V-BPO there have been efforts to develop catalytic protocols with synthetic V-BPO mimics[161-163]. Classical 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[162]. Catalytic protocols with most V-BPO biomimics still contain major disadvantages, such as the use of chlorinated solvents. Moreover, unlike V-BPO which functions most efficiently at near neutral pH [53] most of the model complexes were found to be catalytically active only in acid medium. 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

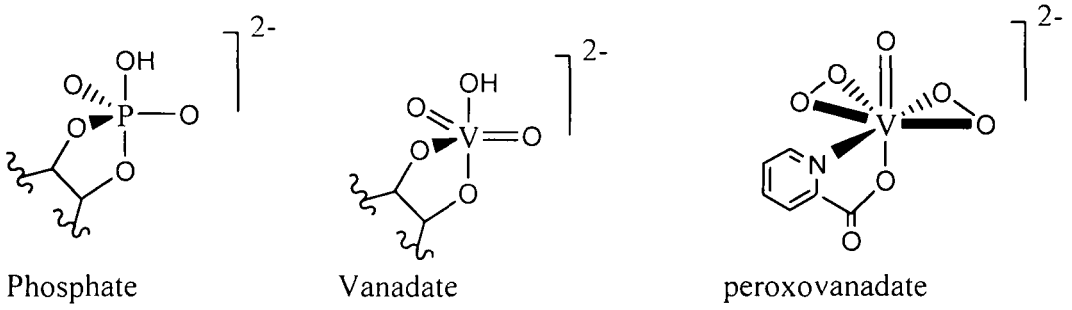
catalysts of the oxidation of halide by hydrogen peroxide which indeed is an important development in this area[59 64 65 67 70] It would be useful to develop peroxovanadium compounds with definite potential for application as safer alternative synthetic catalyst for organic bromination reactions

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

Other very important aspects of pV systems of contemporary interest are their antineoplastic[13 15] and insulin mimetic effects [7 12 17 21 24 25 27 43 45] 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[15] 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[164]

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[165] 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[12] Mechanism by which peroxovanadates mimic insulin is not fully

understood. Vanadium compound, specifically vanadate and peroxovanadate are competitive inhibitor of protein tyrosine phosphatase. A good correlation exists between the PTPase inhibitory abilities of peroxovanadate complexes, their abilities to promote activation of insulin receptor[165], and their *in vivo* insulin mimetic activities. Gresser and Tracey developed an analogy between phosphate and vanadate esters to explain enzyme inhibition by the latter and this analogy can be extended to pV compounds to a certain extent (Fig.1.5).



Phosphate Vanadate peroxovanadate

Fig 1.5 Structural analogy linking phosphate vanadate esters to a peroxovanadate complex

Vanadate act as transition state analog by binding reversibly to thiol group in the catalytic domain , while peoxovanadium complexes oxidize a critical cysteine residue in the catalytic domain. 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. In addition to inhibition of PTPase activity peroxovanadates compounds were also reported as potent inhibitor glucose-6-phosphatase leading to the lowering the hepatic glucose production in diabetic state[166]. Another class of phosphorylase enzyme inhibited by vanadate and its derivative is the alkaline phosphatase[167].

A large number of heteroligand pV complexes have been tested for possible insulin like activities in recent years[168]. However, most of the peroxo compounds are hydrolytically unstable and end with radical formation when subjected to redox processes[7,169] which limits their utility as therapeutic agents.

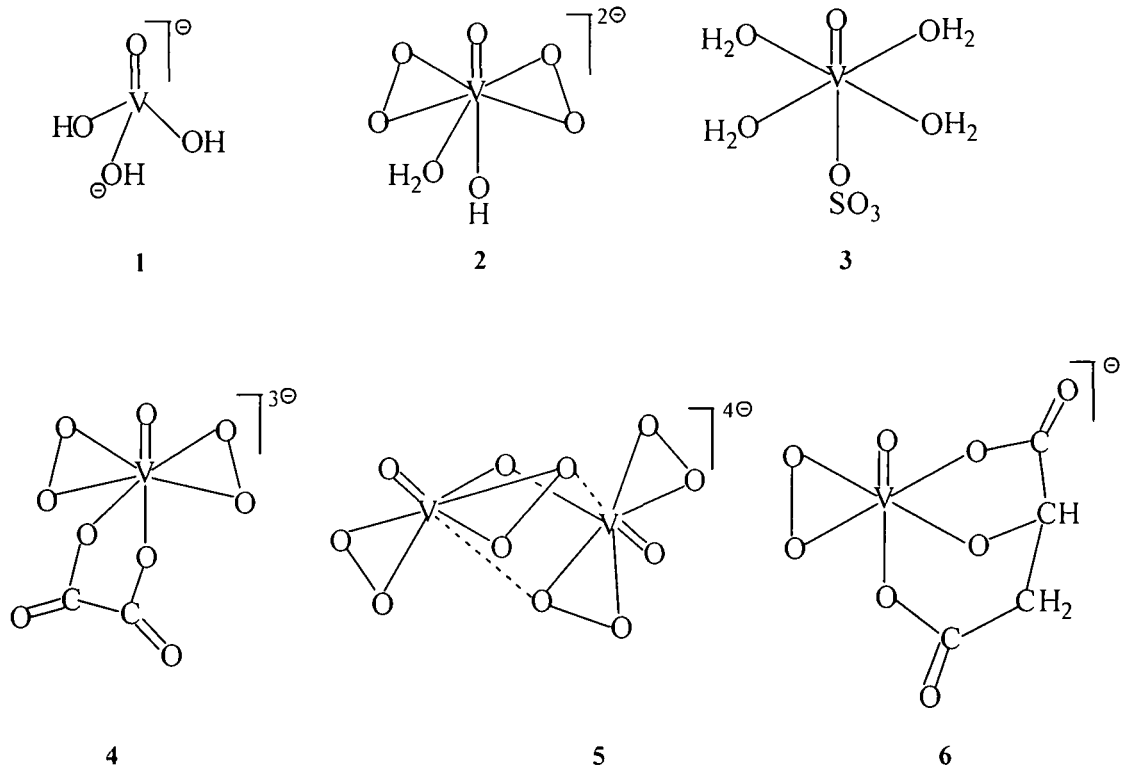


Fig. 1.6 Vanadium compounds of therapeutic importance. These compounds have already been proved to be active in animal tests[6].

There is therefore a continued and intense search for biologically relevant peroxo-vanadium complexes spurred by the pressing need for stable, better absorbed, more efficacious vanadium compounds with therapeutic potential.

It is intriguing in this context that despite the well documented advantages associated with immobilization of active transition metal species on soluble or

insoluble polymer matrices, including enhancement of stability of such systems, development of well-defined peroxovanadates anchored to macroligands have so far received scant attention.

1.5 POLYMER BOUND METAL COMPLEXES

The modification of organic polymers by attaching transition-metal complexes constitutes an active area of current research[170-174,175,176]. Polymers often lack many inconvenient properties of monomeric species, such as lability, volatility, toxicity and odour[170]. Macro complexes have been of interest during the past three decades and have emerged as a new generation of material in the light of their potential applications in diversified fields like, catalysis medicine ecology hydrometallurgy ultra-high strength and superconducting materials liquid crystals electronics device waste water treatment [177-183]. In addition they are used as enzymetic models[184-190].

Anchoring of catalytically active transition metal complexes also received considerable attention due to their potential advantages in practical synthesis viz., (i) easy work up of reaction mixture, (ii) enhanced stability of reagent, (iii) regeneration and reusability of the catalytic agent[170,172]. Much of the stimulation for research in this area has come from the advantages associated with converting selective homogeneous catalysts to heterogeneous polymer supported systems. Various approaches to preparing supported complexes as well as diverse names for the supporting processes such as "heterogenisation of homogeneous complexes", anchoring, attachment, immobilization was proposed.

A macromolecule bearing suitable ligand groups or substituents can interact with metal part (metal ion or metal complexes) by covalent, coordinative, ionic or complex

interactions as schematically shown in *Fig 1.7.*[191-195]. A polymer ligand contains anchoring sites with co-ordinating moieties like N, O, P or S atoms obtained either by polymerization of monomer possessing the coordinating site or by chemical reaction between a polymer and a low molecular weight compound having coordinating ability. The synthesis results in an organic polymer with inorganic functions[196].

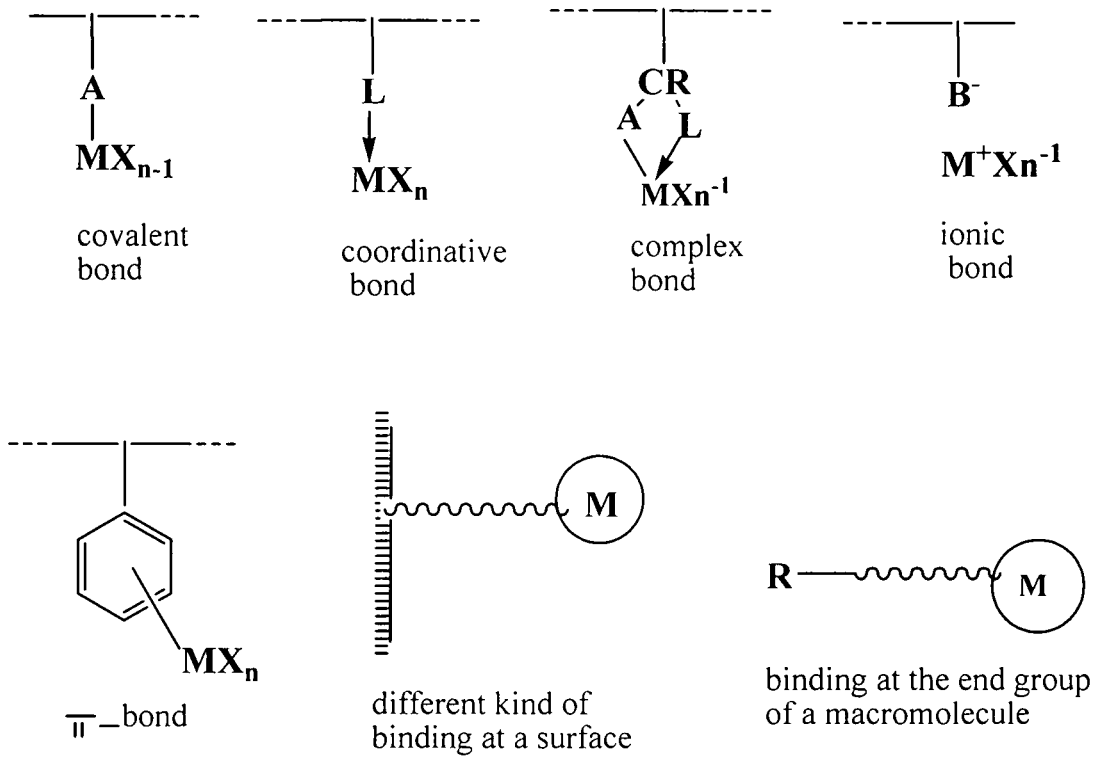


Fig. 1.7. Schematic model of binding of metal ion, metal complexes or π π complexes at macromolecular carriers[191].

Different polymeric materials, natural and synthetic, prepared both by addition and condensation polymerisation, are reported as matrices to anchor complexes (Table 1.4). The interaction of metal complexes MX_n to macromolecule may occur either through monodentate binding or by either intra or inter polydentate binding.

In the case of linear or branched organic polymer the macromolecular metal complexes are soluble in organic solvents and their structure can be identified rather easily. The solubility of bridged macromolecular metal complexes decreases and they are more stable and have less defined structure. The complexes with intermolecular bridge bonds are insoluble and difficult to characterise.

The cross-linked copolymers of styrene with butadiene and divinylbenzene are used most extensively[197]. In this case the functional group for anchoring complexes is attached to the aromatic ring of a polymer chain. A great number of polymer matrices have been obtained where potential anchoring sites are bound to aromatic rings. Another type of cross-linked polymer used for supporting metal complexes is the chloromethylated poly (styrene–co-divinyl benzene)[198-201]. Ion exchange resin is also reported to be used for anchoring cationic and anionic forms of complexes[202].

The catalytic properties of immobilized metal complexes are controlled by a number of factors such as the nature and distribution of attached transition metals ions and the character of the polymer support, together with unreacted functional groups of the polymer after fixation and activation of metal complexes[203]. The use of polymer groups as ligands permits the ligand surroundings to be varied and regulation of the catalytic properties of the complexes becomes possible because of the flexibility of the macromolecular chains and their ability to adopt to various conformations. Depending on the chemical nature of initial components, immobilized complexes can be soluble or insoluble in the reaction mixtures; therefore it is possible to transform homogeneous into heterogeneous catalysts and vice versa, which is a remarkable feature of such systems[203].

Table 1.4 The summary of combinations of metal complexes and macroligands, as well as catalysed reactions most commonly used in practice[204].

Polymer support	Functional group	MX_n	Catalysed reaction
Phosphinated CSDVB	$-PPh_2$	$RhCl(PPh_3)$ $[CODRhCl]_2$ $Rh(CO)_2(PPh_3)_2$ $PdCl_2(PPh_3)$ $RuCl_2(CO)_2(PPh_3)_3$ $RhHCO(PPh_3)_3$ $[COdRhCl]_2$ $Cocl_2(PPh_3$ $Mo(CO)_2(PPh)_2$ $Fe(CO)_4PPh_3$	Hydrogenation Hydroformylation Deuterium/hydrogen exchange Isomerisation Oligomerisation Cyclooligomerisation
CSDVB	Dipy Cp $NH_2CH_2CH_2NH_2$	$Pd(OCOCH_3)_2$ $CpTiCl_3$ $Pt(PhCN)_2Cl$	Hydrogenation
PA	-	Ni(napht)	PhA hydrogenation
PEI/SiO2	NH	Pd^0	nitrobenzene
PE-gr-P4VP	Py	$PdCl_2(PhCN_2)$	p-Nitrochlorobenzene hydrogenation
PMMA	$-COOCH_3$	Pd^0	Nitrocompound hydrogenation
PTFE	-	Au^0	Deuterium/hydrogen exchange
CSDVB	$-NMe_2$	MCl_2(Pt,Ru)	Hydrosilylation
PE-gr-PAAc	-COOH	Co(AcAc)	Cyclohexene oxidation
PAN	$-C\equiv N$	$M(AcAc)_2$(M= Mn,Co)	Ethyl benzeneIsoprpyl benzene oxidation
Polystyrene-co-divinyl-benzene-2-Me-5-VP)	N-	Cu^{2+}	
CSDVB		Co,Ni,VO, CO, Fe, Mn,phtalocyanines	Cyclohexene oxidation
PEG	- OCH_2CH_2 -	MCl_2(M= Co, Mn, Cu) Co^{2+} ,Cu^{2+}	Teralin oxidation H_2O_2 oxidation
Poly(2-vinylpyridine-co-styrene)		Fe^{3+},Co^{2+},phthalocyanines	H_2O_2 oxidation
Phosphate cellulose	$-PO_2OH$	V^{5+},Mo^{5+}	Cyclohexene epoxidation
Chloromethylated CSDVB		Dithiocarbamate, Mo^{5+}	Olefin epoxidation
P4VP	-N	Cu^{2+}	Dialkylphenol oxidation

Polyvinylamine	-NH2	Co^{2+}, Fe^{3+}, Cu^{2+} phthalocyanines	Thiol oxidation
P4VP	-N	Cu^{2+}	Ascorbic acid oxidation
PMMA	C=O	$TiCl_4$, VCl_4	Ethylene polymerisation
PE-gr-PAAI	-OH	$Ti(OBu_4)_4$	Ethylene polymerisation
PE-gr-P4VP	-N -N	$TiCl_3$ $CoCl_2$	Stereospecific propylene polymerisation 1,4-cis-butadiene polymerisation
Copolymer of styrene and acrylic acid	-COOH	$Ni(napht)_2$	1,4-cis-butadiene polymerisation
PE-gr-PAAc	-COOH -COOH	$Ni(CH_3COO)_2$ Ni^{2+}, V^{4+}	Ethylene polymerisation "Relay-race" ethylene copolymerisation
Triple copolymer of ethylene propylene and P4VP	-N	$CoCl_2$, $NiCl_2$	Butadiene di and oligomeerisation
PS	-	$AlCl_3$,$TiCl_4$	Styrene and ethyl styrene polymerisation
PE-grPAAI	-OH	$MoCl_5$,WCl_5,Cu^{2+}	PhA polymerisation
CSDVB-P4VP	-N	Cu^{2+}	Oxidative polycondensation of phenols
PE-gr-PAAc	-COOH	$Co(OCOCH_3)_2$	Phenol formaldehyde oligomers solidification
CSDVB	Cp	$CpCo(CO)_2$	Fischer-Tropsch synthesis
PEI	-NH	$RhCl_3$	Methanol carbonylation
PVAI	-OH	Cu^{2+}	2,4 dinitrophenyl acetate hydrolysis
CSDVB		$TiCl_4$	Etherification Alkylation, acetalisation
Copolymer of styrene and AAc	-COOH	Cp_2TiCl_2	Reduction of nitrogen to ammonia
Thioacetalderivatives of poly(4-amino-styrene)	$-CH_2$ $-NH_2$	$-Mo(NMe_2)_4$ -	Nitrogen reduction Photocatalytic hydrogen formation
PEI	-NH	Rh^{3+}	Formation of H_2 from H_2O
CSDVB	-	Mg,Mn,Fe,Ru,P thalocyanines	Isomerisation of quadricycalne to norbornadiene
Poly[$Ru(DiPy)_3^{2+}$	-	-	Chemically modified electrodes
P4VP/Carbon	-N	Cu^{2+}	Electrochemical reduction of O_2 to H_2O
Poly[$Ru(Dipy)_3^{2+}$-4VP/Pt	-	-	Photodiodes (Chemically modified photoelectrods)

Polymer-immobilized catalysts are closer to homogeneous catalysts in chemical character. Since they are usually synthesized from soluble metal complexes, they show similarity in chemical behavior with such complexes. Immobilisation of metal complexes usually increase their efficiency and stability[170]. In fact, immobilised catalysts combine the main features of homogeneous, heterogeneous and enzymatic catalysts.

A branch of chemically active polymers is "redox polymers", which are polymers that undergo reversible redox processes. These are mostly metal containing polymers where co-ordination chemistry of the redox active site plays a major role. Attempts to produce heterogenized reduction systems began with the invention of the column reactor by Jones in 1889[170]. By 1963 redox active polymers had found application to the quantitative redox of metal ions and organic substrates[205,206] via the columnar procedure devised by Jones. Nowadays a plethora of oxidation and reduction are performed by redox polymers. Catalysts of the Zeigler-Natta type such as complexes of Ti, Zr, Hf, V, Mo, Co, Ni, Cr etc immobilized on polymers in combination with organoaluminium compounds are active catalysts for unsaturated substrate reductions[170,207].

Data on oxidation of various types of substrates by dioxygen in the presence of polymer-supported catalysts have been published viz., oxidation of catechol amines[208], hydrocarbons[209-212], selective epoxidation[213-217], hydroformylation of olefins[218] and etherification[219], etc. Undoubtedly, oxygen from the air or dilute solutions of H_2O_2 are ecologically most suitable oxidants for large-scale processes, and metal ions can be used as catalysts[220]. In terms of polymer supported system the most widely investigated are W(VI)/H_2O_2, V(V)/ROOH, Mo(VI)/ROOH[221]. Reports are available on catalytically active O_2 bound macromolecules consisting of metals

viz., Cr, Mn, Fe, Co, Mo[221]. Features of the polymeric ligand effect the kinetics and even directions of these reactions. For example macrocomplex of Co^{2+}-polyethyleneimine(PEI) can reversibly bind oxygen with formation of a μ-peroxo adduct stable in aqueous solution at room temperature[222], In addition to Co macrocomplexes, immobilized Cu^{2+} compounds are most often used for O_2 activation[223]. Recent developments in biomimetic redox catalysis have shown that enzymes are but biological polymeric catalysts which can be mimicked, and that the processes of nature can be modeled and understood.

1.6 MACRO COMPLEXES AS ENZYMATIC MODEL

Immobilized complexes can be considered as models of biological catalysts because they can carry out multicentre activation of a substrate which is characteristic for metal enzyme catalysis[184,190]. This resemblance may also be explained by the molecular mobility both of proteins and macroligands in synthetic protein analogues. Since in metal enzymes metal ions are incorporated into the structure of biomembranes they may be considered as immobilized complexes incorporated into swollen polymer matrices.

Metal polymers are interesting as models of catalase, peroxidase, proteolytic and other enzymes[172]. Liquid-phase decomposition of hydrogen peroxide is a convenient model for various redox processes including enzymatic ones. The most important success in activation of molecular oxygen was achieved for immobilized complexes of Fe^{3+}, Mn^{3+} and Co^{3+} with porphyrins and pthalocyanines[185-187]. These are studied as model systems of metal enzymes for cytochrome P450, myoglobin and

hemoglobin[185-187]. Polymer ligands, like protein globins, prevent dimerization of active complexes through matrix isolation.

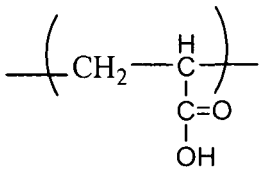
It has been shown by others that some polymer-metal chelates exhibited catalase like behaviour, whereas metal complexes of the monomer from which the polymer was derived were found to possess little or no activity[170]. Enzyme catalase is an effective catalyst for H_2O_2 decomposition. There are many heterogenized enzymetic systems, obtained by immobilization of catalase on synthetic polymers (biomimetic polymers), which decompose H_2O_2 to H_2O and O_2, for instance derived from PEG[189]. Catalase-type activity of Cu^{2+} complexes immobilized on CSDVB modified by Schiff bases was studied comprehensively[172].

1.7 METAL COMPLEXES SUPPORTED ON SOLUBLE POLYMER

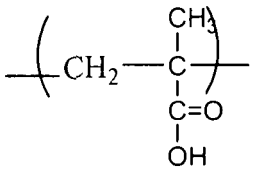
Apart from the insoluble polymeric matrices used as supports, increasing attention is now being paid to the concept of attaching metal complexes to the soluble polymers[224-231]. One of promising trends in catalyst design is the use of water soluble macromolecular metal complexes. The use of environmentally more benign solvents such as water is now a subject of considerable interest in the context of green chemistry[232]. Despite the well known advantage of insoluble supports, there are several shortcomings in the use of these resins since the catalyst often suffers from lowered catalytic activity and enantioselectivity after it has been anchored onto a polymer[233-237]. This is often attributed to limited accessibility of the catalysts active sites due to the heterogeneous nature of the reaction. Also, the irregular achiral structure of the polymer-support may create microenvironments at the catalytic sites that are very different from that of the homogeneous catalyst. A way to over come this

problem is to use low molecular weight soluble linear polymers as supports[238-242]. Moreover, the reactivity and selectivity of the catalyst anchored on the soluble support can equal that of its unsupported homogenous analogue. Recovery of the polymer supported catalyst can be achieved by temperature or solvent-induced precipitation followed by filtration. Non crosslinked polymers can be completely dissolved in the reaction medium. In this case, transition metal complexes attached to such polymers is essentially a homogeneous catalyst with macromolecular ligands.

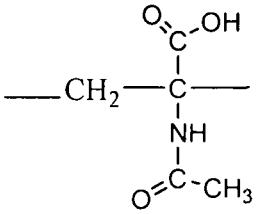
The use of soluble polymers to recover catalyst and ligands in synthetic approaches to peptide and oligopeptide synthesis were developed by Merrifield and Letsinger in the 1960's[243,244]. These discoveries revolutionised the synthesis of biomolecules[245]. They provided impetus for research in industrial and academic laboratories that was directed toward developing immobilized or heterogenized homogeneous catalysts. The first example where a soluble polymer was used as an alternative to a cross-linked insoluble polymeric resin to support a chiral ligand, was reported in 1976 by Bayer and Schurig[246]. A DIOP (4,5-bis(diphenylphosphinomethyl)-2,2-dimethyl-1,3-dioxolane) ligand was attached to a linear polystyrene. The resulting polystyrene-bound version of DIOP was allowed to react with $HRh(CO)\text{-}(PPh_3)_3$, and the resulting polymer-bound Rh complex was used to hydroformylate styrene. A popular type of soluble macromolecular metal complexes used in catalysis is modified poly(ethylene oxide) catalyst for



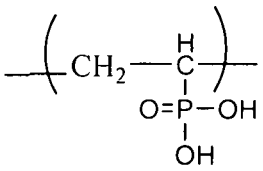
Polyacrylic acid
(PAA)



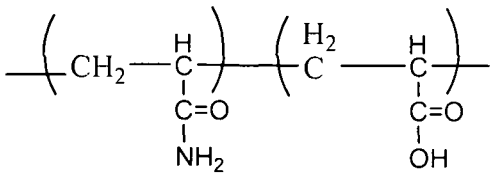
Polymethacrylic acid
(PMA)



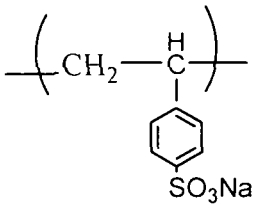
Polyacetylamino acrylic acid
(PAAA)



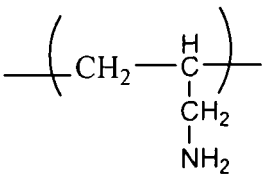
Polyvinylphosphonic acid
(PVPhA)



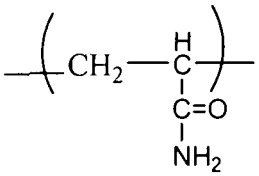
Polyacrylamide-co-acrylic acid
(PAm-co-PAA)



Poly(sodium 4-styrenesulfonate
(PSS)



Polyallyamine
(PALA)



Polyacrylamide
(PAm)

Fig. 1.8 Some water soluble polymer used for metal ion interaction

hydroformylation[247-250], wacker oxidation[251,252], hydroxylation of aromatic compounds[253,254] and epoxidation[255]. Polycarboxylic acid and its derivative are often used to synthesise soluble ligands and complexes. Besides polyacrylic acid, poly(pentenoic acid) has also been used to produce phosphine containing ligands and Rh complex which are active in hydrofromylation of 1-alkenes[256]. Polyacrylamide modified with optically active phosphine containing ligands and Rh catalyst were used for asymmetric hydrogenation of prochiral amides[257-259]. Among the first soluble macromolecular metal complexes to be used as catalyst were modified linear polystyrenes[260-264].

In addition to the importance of soluble macromolecular metal complexes in catalysis there has been considerable contemporary interest in development of pharmaceutical formulations consisting of water soluble polymers[265-268]. Polymers as drug carriers have been investigated to achieve efficient delivery of anticancer agents to tumor cells. The binding of active drug molecule including low molecular weight metal complexes to macromolecular carriers is of considerable importance due to its overcoming of limitations, such as short circulation residence time and powerful toxic side effects. A few linear and water soluble polymeric platinum complexes have been developed in recent years which are reported to be useful anticancer agents[269,270]. The use of polymer–metal ion adducts open new strategies in biological applications. In most cases, the water-soluble polymer–metal complexes exhibit a cationic polyelectrolyte behavior in aqueous solution. For this reason they are potentially biologically active compounds. In this context, Lee and Rashidova[271] studied the biological activity, toxicity, immunological response and the pharmacokinetics of several polymer metal complexes of N-vinylpyrrolidone and derivatives of N-2-hydroxypropylmethacrylamide with transition metals. The effect of the macroligand

type, polyacid behavior, and the comparison of the biological properties between the polymer–metal complexes and the polymer bases were performed. Nandi and co-workers[272] studied the bactericide activity of metal ions and polymer–metal complexes with Co^{2+}, Zn^{2+} and Cu^{2+} whereas Nonaka and co-workers[273] studied the bactericide activity for *E. coli* and *S. aureus* of resins containing the triethylamine and thiols as side groups and the metal ions Ag^{+}, Cu^{2+} and Zn^{2+}.

Notwithstanding the enormous progress in the field of metal containing polymers, we are yet to come across literature report dealing with design, synthesis or use of peroxometal compounds anchored to soluble polymers. A perusal literature shows that few peroxometal systems[274-276] including one pV compound[277], PSK[$VO(O_2)_2$(L) where L=2pybmz and PS= chloromethylated polystyrene-co-divinylbenzene, supported on insoluble crosslinked polymer were synthesized which were found to exhibit good activity as catalytic or stoichiometric oxidant in organic oxidation.

1.8 RESEARCH OBJECTIVES

From the above non-exhaustive discussion it is apparent that synthesis and characterization of well defined peroxo metal complexes attached to water-soluble polymer matrices and assessment of their stability, redox and biochemical properties constitute a fascinating and rewarding area of investigation.

Major objectives of the present research programme are as follows:

(i) To develop synthetic routes to novel peroxo complexes of vanadium(V) by anchoring peroxovanadate species to water soluble polymer matrices and to characterize them.

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

(iii) To explore biochemical properties of the polymer-bound pV compounds, particularly involving their effect on phophorylase enzyme and interaction with catalase.

(iv) To investigate the antibacterial behavior of the free monomeric as well as polymeric perxovanadate complexes towards the gram positive *S. aureus* and gram-negative *E. coli* bacteria.

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

(vi) To undertake theoretical investigation on structure of the peroxobridged dinuclear vanadium complexes by using Density Functional Theory.

(vii) To study the reactivity of active site of the complexes by reactivity descriptor with an aim to understand the mechanism of peroxidative bromination reaction known to be brought about by these complexes.

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 either published or accepted for publication and the rest is under communication.

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CHAPTER 2

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, polyacrylic acid sodium salt, poly(sodium 4-styrene sulfonate) and poly(sodium styrenesulfonate-*co*-maleate) (Sigma-Aldrich Chemical Company), amino acids, sodium and potassium vanadates, cysteine, sodium thiosulphate, potassium persulphate (CDH);, potassium iodide, potassium hydrogen phosphates (E. Merck, India); Tungstic acid, dithionitrobenzoic acid (DTNB), glutathione (GSH) (Himedia laboratories, Mumbai,India); Alkaline phosphatase from rabbit intestine, p-nitrophenyl phosphate (p-NPP), glycyl-peptides, phenol red, catalase, (Sigma Alderich Chemicals Company Pvt. Ltd.); Luria Agar, Luria Broth (HiMedia Laboratories, India), Potassium dihydrogen phosphates, sodium and potassium hydroxides, aniline, nitroanilines, aminophenols, quinol, 2-methoxytoluene, acetone, diethyl ether, ethyl acetate, petroleum ether 40°-60°C (SD fine chemicals). Sodium diperoxovanadate (NaDPV) was prepared by the method described earlier[1].

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

2.2 ELEMENTAL ANALYSES

2.2.1 Vanadium[2]

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

2.2.3 Peroxide[3-5]

2.2.3 1 Permanganometry[3]

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

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

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

2.2.3.2 Iodometry[4]

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

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

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

2.2.3.3 By standard Ce(IV) solution[5]

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.

2.2.4 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 by Perkin Elmer 2400 series II at the Department of Chemical Sciences, Tezpur University

2.2.5 Sodium

Sodium contents were determined by Atomic Absorption Spectroscopy.

2.3 PHYSICAL AND SPECTROSCOPIC MEASUREMENTS

2.3.1 pH measurement

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

2.3.2 Molar conductance

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

2.3.3 Magnetic susceptibility

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

2.3.4 Electronic spectra

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

2.3.5 Infrared (IR) spectra

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

2.3.6 ^{1}H-NMR spectra

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

2.3.7 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).

2.3.8 Thermogravimetric analysis

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

2.3.9 Surface morphology analysis by Scanning electron microscope

The SEM characterization was carried by using the LEO 1430 VP scanning electron micrograph attached with energy dispersive X-ray detector.

2.3.10 Computational calculation

Density functional studies was studied by $DMol^3$ program, Materials Studio 2.0, Accelrys Inc., San Diego, CA, USA

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CHAPTER 3

New polymer bound peroxo and heteroligand peroxo complexes of vanadium(V): synthesis, characterization and stability

3.1 INTRODUCTION

The burgeoning interest in peroxovanadium (pV) compounds, as emphasized in the literature and highlighted in the introductory chapter, is based on a variety of reasons including their favourable catalytic properties[1,2] and biochemical relevance[1-5]. Much research has been performed on the potential usage of compounds of vanadium and its peroxo derivatives as therapeutic antidiabetic agents[4,5], their affinity as inhibitor or activator of enzyme functions [3,6,7], as well as versatile redox catalysts[6-8]. However, most of the synthetic pV compounds tested for their various biochemical effects suffer from the disadvantage of being hydrolytically unstable which limits their pharmacological potential[4-6]. Consequently, efforts to generate stable pV systems possessing appropriate bio-relevant characteristics continues unabated.

Activation and binding of dioxygen by polymer anchored transition metal is interesting from the view point of designing effective redox catalysts and modelling of complex processes in bio-systems[9,10]. A polymeric support is also likely to impart stability to the anchored complex species. Although a variety of pV complexes have been synthesized in

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recent years there is a paucity of information on well-defined peroxovanadates where a macromolecule such as a functionalized polymer provides the hetero-ligand environment[11-14].

We have therefore endeavoured to develop pV systems with enhanced stability and oxidative ability by anchoring active pV to polymer supports. Our primary goal has been to prepare water soluble macrocomplexes containing peroxovanadate with biologically relevant properties, in the hope that these materials may also function as useful oxidants in organic oxidation including mild oxidative bromination.

The utility of water soluble polymers as supports in organic chemistry and biology, as mentioned in Chapter 1, is increasingly being recognized in recent years[15,16]. One of the significant features of a soluble polymer supported reagent is the facility of product synthesis and characterization afforded by the soluble support due to the advantages of homogeneity offered by it. There has been a continuous upsurge in interest in metal containing polymeric materials in general, due to their tremendous potential in diverse fields like catalysis[17,18], liquid crystals[19], biosensors[20], drug delivery systems[21,22] and a host of other biomedical applications[23]. It is apparent after reviewing the literature that although a few peroxometal systems including one pV compound, supported on insoluble cross-linked polymers were prepared recently which were reported to exhibit good activity as catalytic or stoichiometric oxidant in organic oxidations, none of the known soluble polymers have been explored till date, for use as polymeric support to obtain peroxo metal species in macro ligand environment.

The polymeric ligands chosen for the present study required to possess the ability to form stable complexes with peroxo-vanadium(V) moiety. The task of establishing chemically robust metal-polymer linkage which will remain intact in solution thereby preventing the leaching of the metal is challenging particularly in case of the regeneration of supported

catalytic species. The information derived from synthesis of unbound hetero-ligand peroxovanadates have been particularly useful in identifying co-ordinating groups which will help in retaining the anchored complex species after completion of the reaction cycles. Pertinent here is to mention that for almost a decade our group has engaged its attention to devise synthetic strategies and to make systematic studies on redox and biochemical properties of peroxo-metal compounds with physiologically relevant molecules acting as ancillary ligands[24-28]. We selected, for the purpose of this investigation, polymers viz., poly(acrylate), poly(methacrylate), poly(sodium 4-styrene sulfonate) and a co-polymer poly(sodium styrene sulfonate-*co*-maleate), as supports owing to the advantageous features associated with these polymeric species such as their convenient method of preparation or commercially availability, water solubility, chemical stability, presence of appropriate functional groups for easy attachment of active metal complexes, which are some of the basic requisite features for a polymer to possess in order to serve as soluble support. In addition, there has been considerable contemporary interest in development of pharmaceutical formulations consisting of acrylic acid and styrene sulfonic acid based polymers and their derivatives[29-32]. Polymers such as polyacrylate and polystyrenesulfonate have pH dependent solubility and have been used to advantage to prepare catalytically active complexes that can be dissolved by adjusting the pH of a solution.

The present chapter reports the synthesis and physico chemical characterisation including thermal analysis of a series of water soluble polymer bound peroxovanadium complexes of the type $[V_2O_2(O_2)_4(carboxylate)]$-PA [PA = poly(acrylate)] (**PAV**) **[3.1]** and $[VO(O_2)_2(carboxylate)]$-PMA [PMA = poly(methacrylate)] (**PMAV**) **[3.2]** $[VO(O_2)_2(sulfonate)]$-PSS [PSS = poly(sodium 4-styrene sulfonate)] (**PSSV**) **[3.3]** and $[V_2O_2(O_2)_4(carboxylate)VO(O_2)_2(sulfonate)]$-P(SS-*co*-M) [P(SS-*co*-M)= poly(sodium styrene

sulfonate-*co*-maleate)] (**PSS-co-MV**) [**3.4**]. The results of studies in nature and stability of the compounds toward decomposition in solution are also reported in this chapter.

Also included in this chapter is the synthesis and characterisation of hitherto unreported triglycine peroxovanadium complexe, $Na[VO(O_2)_2(triglycine)].3H_2O$ (**PV1**)[**3.5**]. Despite the variety and number of heteroligand pV complexes that has been synthesized in recent years[3,33-34] and the intense biological work and solution studies carried out on interaction of vanadates with biogenic species viz., amino acids, peptides and proteins[24-28,35-41] reports regarding the well characterised synthetic peroxovanadium complexes with coordinated peptides are very few[24-28 41]. Peptideds 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. Since one of the aims of the present investigation was to examine and compare some biological properties of free and polymer bound peroxovanadates, it was felt necessary to gain an access to pV compounds with a tripeptide as heteroligand possessing appropriate characteristics of stability and solubility.

3.2 EXPERIMENTAL SECTION

3.2.1 Synthesis of $[V_2O_2(O_2)_4$(carboxylate)]-PA (PA= Sodium polyacrylate) (PAV) [3.1], $[(VO(O_2)_2$ (carboxylate)]-PMA (PMA= Sodium polymethacrylate) (PMAV) [3.2], $[VO(O_2)_2$(sulfonate)]-PSS [PSS = Poly(sodium styrene 4-sulfonate)] (PSSV) [3.3] and $[VO(O_2)_2$(sulfonate)$V_2O_2(O_2)_4$(carboxylate)]-P(SS-co-M) [P(SS-co-M)= poly(styrene sulfonate-co-maleate)] (PSS-co-MV) [3.4]

The procedure adopted for synthesis is common to all the four macromolecular complexes. This consisted of gradual addition of 12 ml H_2O_2 (30% solution, 105.84 mM) to a mixture of 0.36 g (2.0 mmol) V_2O_5 and 1.5 gm of respective polymer (for compound **3.1** and **3.2**) and 0.25 g (1.3 mmol) V_2O_5 and 2ml and 1.0 gm of respective polymer (for compound **3.3** and **3.4**) . Keeping the temperature below 4 °C in an ice bath, the mixtures were stirred for *ca.* 30 minutes until all solids dissolved. The pH of the solution was recorded to be *ca.* 3. Concentrated sodium hydroxide (*ca.* 8 M) was added drop wise with constant stirring to raise the pH of the reaction medium to *ca.* 6. On adding pre-cooled acetone (*ca.* 50 ml) to this mixture under vigorous stirring a yellow colored pasty mass separated out. After allowing it to stand for 20 min in the ice bath, the supernatant liquid was decanted and the residue was treated repeatedly with acetone under scratching until it became microcrystalline solid. The product was separated by centrifugation, washed with cold acetone and dried in vacuo over concentrated sulfuric acid. In the solid state these compounds were found to be stable for several weeks stored dry in closed containers at $\leq 30\ ^0C$.

3.2.2 Synthesis of peroxovanadate complex, $Na[VO(O_2)_2(triglycine)].3H_2O$ (pV1)[3.5]

Solid V_2O_5 (0.25 g, 1.37 mmol) was mixed with triglycine in a 250 ml beaker maintaining molar ratio of V: triglycine as 1:1 to which 12 ml of 30% H_2O_2 (105.84 mmol) was added gradually with constant stirring. Keeping the temperature below 4°C in an ice bath, the mixture was stirred for *ca.*15 minutes until all solids dissolved. At this stage the pH of the solution was recorded to be *ca.*2. Dilute NaOH solution (0.1 M) was added drop wise to the solution with constant stirring to raise the pH of the reaction medium to *ca.*5.5. On adding pre-cooled acetone (*ca.* 50 ml) to this mixture under vigorous stirring a yellow

coloured pasty mass separated out. After allowing it to stand for about 15 minutes in the ice bath, the supernatant liquid was decanted, and the residue was treated repeatedly with acetone under scratching until it became microcrystalline solid. The product was separated by centrifugation, washed with cold acetone and dried in *vacuo* over concentrated sulfuric acid. In solid state these complexes were found to be stable for several months stored dry at <20°C.

3.2.3 Elemental analysis

Quantitative estimations of vanadium, peroxide, carbon, hydrogen, nitrogen and sodium 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

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

3.2.5 Stability of the complexes in solution

Stability of the compounds in distilled water at pH *ca.* 6, which is the natural pH of the compounds in solution, was studied by estimating the peroxide content in aliquots drawn

from the respective solution of the compound **PAV[3.1]** (0.11 mg/ml) or **PMAV[3.2]** (0.14 mg/ml), **PSSV[3.3]** (0.23 mg/ml) or **PSS-co-MV[3.4]** (0.192 mg/ml) or **PV1[3.5]** (0.1 mM) compound at different interval of time by the method described in Chapter **2**. As a measure of stability of the compounds in solution changes in absorbance of their electronic spectral band at *ca.* 320 nm at ambient temperature were recorded at 30 min gap for a period of 24 h. Moreover in order to examine the stability for compound **pV1[3.5]** molar conductance in was recorded at ambient temperature for a period of 24 h.

3.3 RESULTS AND INTERPRETATION

3.3.1 Synthesis and characterization

3.3.1.1 Synthesis

The methodology for the successful synthesis of the polymer anchored peroxovanadates **3.1-3.4** was based on the reaction of V_2O_5 with H_2O_2 and respective polymeric ligand in an aqueous medium. In the present study, the macromolecular peroxovanadium complexes **3.1-3.4** were isolated at pH value *ca.* 6. The alkali used to raise the pH of the reaction solution also served as source of counter cation for the complex anions. The procedure included other essential components such as maintenance of required time and temperature at $\leq 4\ ^0C$ and limiting water to that contributed by 30% H_2O_2 and alkali hydroxide solution. Solvent precipitation is a valuable and general way to isolate soluble polymer bound compounds. The compounds were isolated by addition of acetone which facilitated the precipitation.

The yellow micro-crystalline pV(triglycine), **pV1[3.5]** was isolated from the reaction of V_2O_5 with H_2O_2 and the tripeptide ligand at near neutral pH of *ca.* 5.5. The factors such as sequence of addition of the reactants as well as maintenance of required reaction time and temperature at $\leq 4\ ^0C$ were found to be equally important for achieving the desired synthesis of the compound.

3.3.1.2 Characterization

The elemental analysis data of the compounds **3.1 – 3.5** (Table 3.1) indicated the presence of two peroxide groups per metal centre. The vanadium loading on the compounds based on elemental analysis and confirmed by EDX spectral analysis was found to be 2.07 mmol for **PAV[3.1]**, 1.59 mmol for **PMAV[3.2]**, 1.17 mmol for **PSSV[3.3]** and 1.56 mmol **PSS-*co*-MV[3.4]**, respectively per gram of polymeric support.

The presence of two peroxide groups and one peptide ligand per vanadium centre were evident for the monomeric compound **pV1[3.5]** from the elemental analysis data, which could be fitted with the formulation of the complex species as $Na[VO(O_2)_2(triglycine)].3H_2O$. Results of the molar conductance measurement at ambient temperature revealed 1:1 electrolytic nature of **pV1[3.5]** (129 $\Omega^{-1}cm^2mol^{-1}$) lending further evidence in support to the formulation. The title compound is diamagnetic in nature as was evident from the magnetic susceptibility measurement in conformity with the presence of V(V).

3.3.1.3 SEM and Energy Dispersive X-ray (EDX) Analysis

Scanning electron microscopy was used to investigate the morphological changes occurring on the surfaces of the polymers after loading of the peroxovanadates to the polymer chains. In contrast to the smooth and flat surfaces of the pure poly(acrylate) poly(methacrylate), polystyrene sulfonate, polystyrenesulfonate-co-maleate polymers, the

Table 3. 1

Analytical data of the polymer-bound peroxovanadate complexes **3.1 - 3.5**

Compound	% Found from elemental analysis (% obtained from EDX spectra)					Metal ion loading [a]
	C	H	Na	V	O_2^{2-}	(mmol g^{-1} of polymer)
1. **PAV [3.1]**	22.31	6.29	-	10.57	13.2	2.07
	(23.49)	-	(13.48)	(9.51)	-	
2. **PMAV [3.2]**	28.77	7.20	-	8.13	10.11	1.59
	(29.12)	-	(11.4)	(7.9)	-	
3. **PSSV [3.3]**	12.52		-	6.0	7.0	1.17
	(11.63)	3.24	(9.21)	(5.48)	-	
4. **PSS-*co*-MV[3.4]**	23.52		-	8.0	10.0	1.56
	(24.15)	3.71	(16.21)	(7.5)	-	
5. **pV1 [3.5]**	19.50	2.57	5.65	13.74	17.50	

[a] Metal ion loading = $\frac{\text{Observed metal \\% } \times 10}{\text{Atomic weight of metal}}$

surfaces of the polymer anchored complexes exhibited considerable roughening (*Fig 3 1* and *3 3*) Energy dispersive X-ray spectroscopy of the compounds, which provides *in situ* chemical analysis of the bulk, clearly showed V, C, O and Na as the constituents of the anchored complexes (*Fig 3 2* and *3 4*) Moreover, the EDX spectral data obtained on the composition of the compounds were in good agreement with the elemental analysis values

3.3.1.4 IR and electronic spectral studies

The significant features of the IR spectra of the polymer bound peroxo complexes **3.1 – 3.5** are summarized in Table 3 2 The presence of side-on bound peroxo ligand in the compounds, was evident from the observance of the characteristic ν(O-O), $\nu_{asym}(V\text{-}O_2)$ and $\nu_{sym}(V\text{-}O_2)$ modes, in the vicinity of *ca* 870, *ca* 610 and *ca* 530 cm^{-1}, respectively[43] The spectra enabled clear identification of ν(V=O) near 960 cm^{-1} arising from terminally bonded V=O group[44] The spectral pattern attributable to peroxovanadate moiety compared very well with the one observed for free DPV

Characteristic differences were evident between the spectral pattern originating from polymer-metal complexes and spectra of the free polymers *Fig 3 5* and *3 6* Previous investigations on the interaction of poly(acrylate) and metal ions reveal that the frequency difference between the symmetric and antisymmetric stretches ($\Delta\nu = \nu_{asym} - \nu_{sym}$) of the carboxylate group in the polymer coordinated complexes compared to the free polymer can

Table 3.2.

Infrared spectral data of the peroxovanadate complexes **3.1-3-5**

Compound	IR peaks (cm^{-1})				UV peak (nm)
	$\nu(V\text{-}O_2)$		$\nu(V\text{-}O_2)$	$\nu(O\text{-}O)$	$\nu(V{=}O)$
1. **PAV [3.1]**	525	618	872	969	322
2.**PMAV [3.2]**	526	637	875	966	320
3.**PSSV [3.3]**	525	618	870	968	320
4. **PSS-*co*-MV [3.4]**	530	617	873	969	324
5. **pV1 [3.5]**	539	620	869	952	324
3. **DPV**	522	602	877	935	325

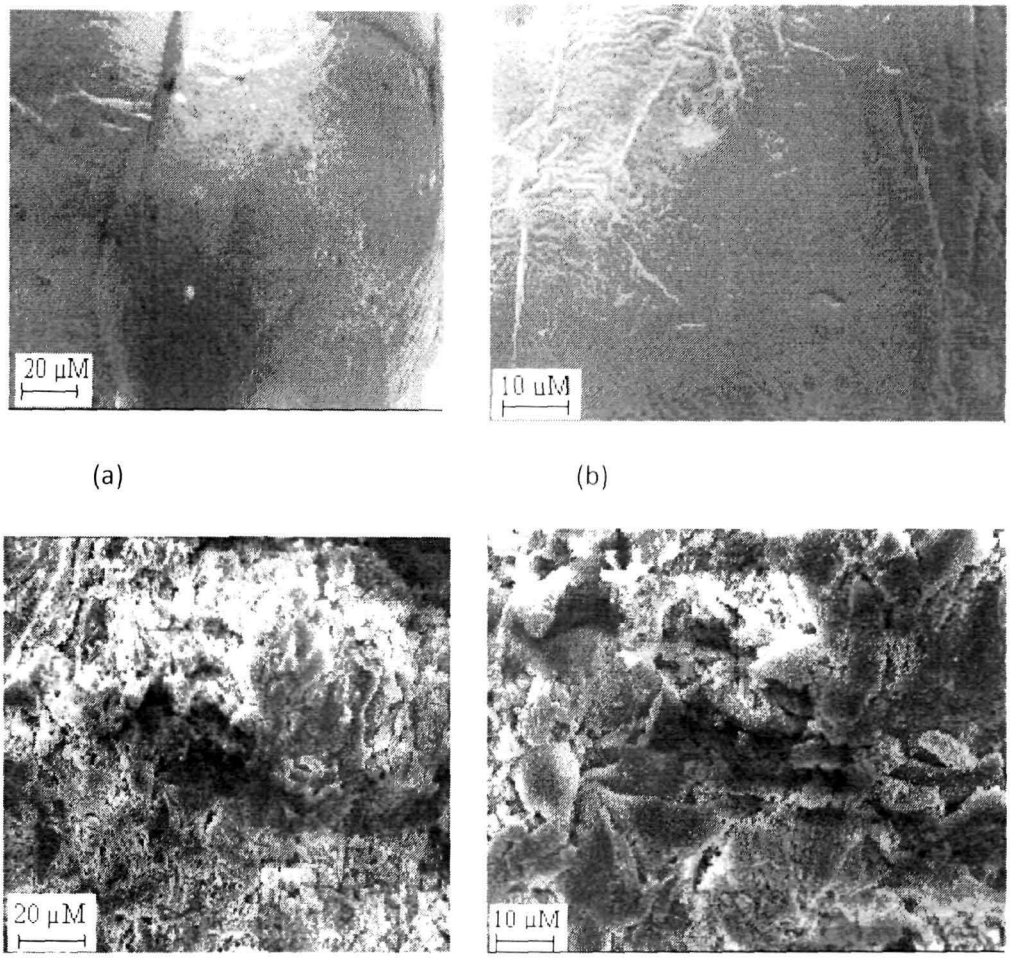

Fig.3.1. Scanning electron micrographs of (a) Sodium poly(acrylate) (b) Sodium poly(methacrylate) (c) **PAV** and (d) **PMAV**.

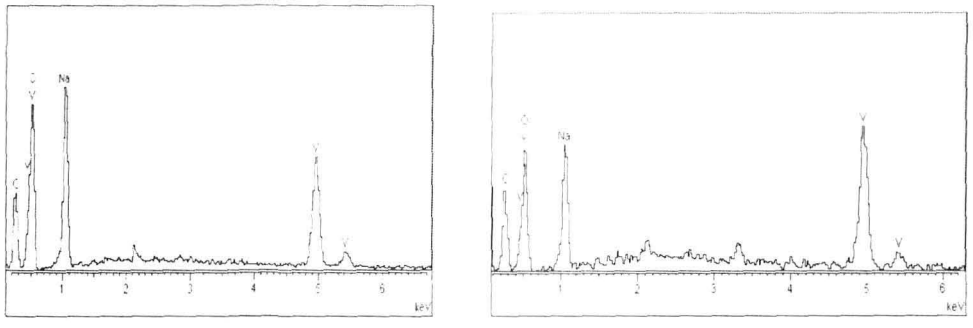

Fig. 3.2. EDX spectra of (a) **PAV** and (b) **PMAV**.

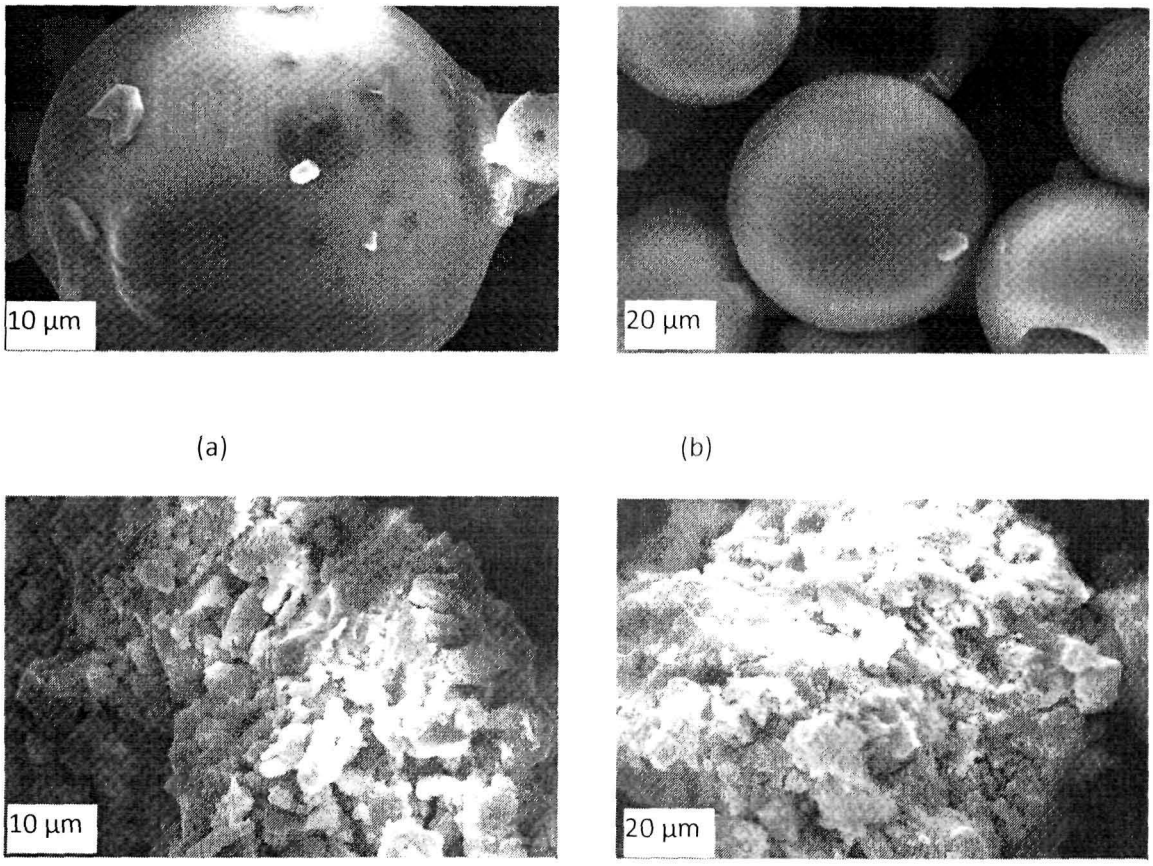

Fig 3.3. Scanning electron micrographs of (a) **PSS** (b) **PSS-*co*-M** (c) **PSSV** and (d) **PSS-*co*-MV**

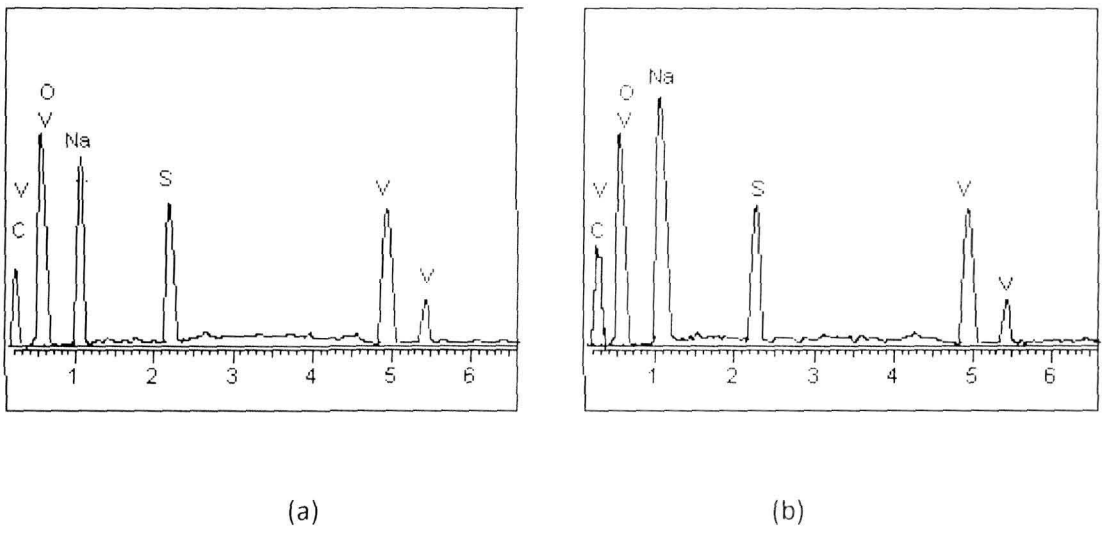

Fig 3.4. EDX spectra of (a) **PSS-*co*-MV** and (b) **PSSV**.

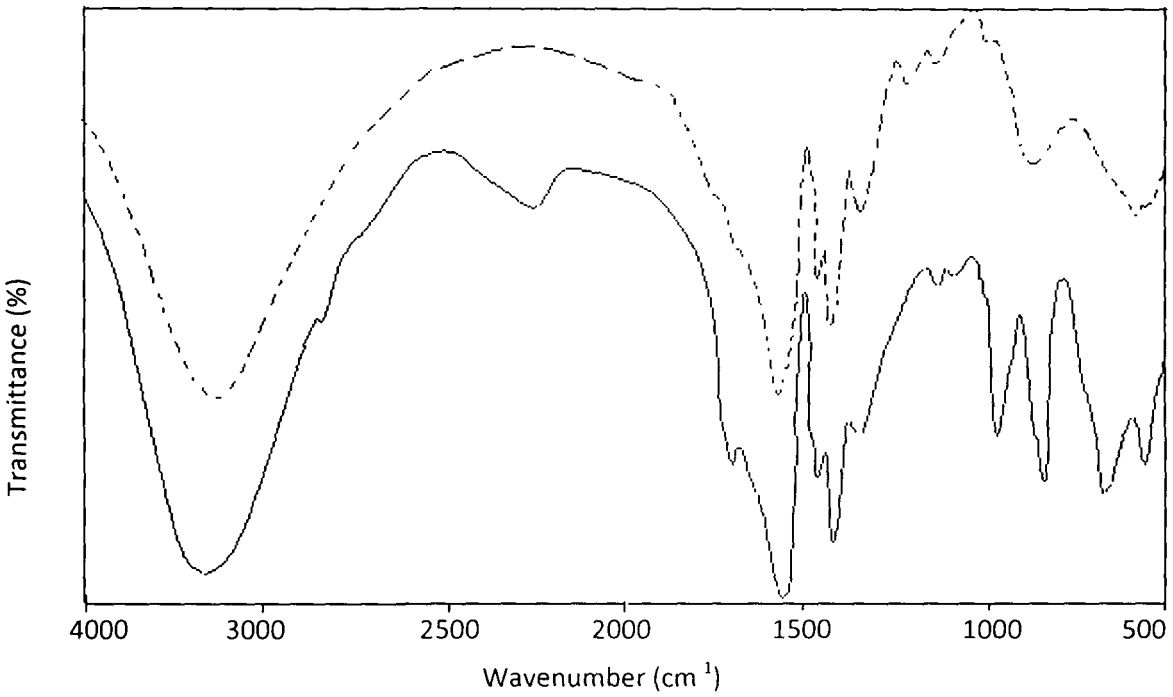

Fig. 3.5 IR spectra (**PAV**) [**3.1**] (solid line) solid line and **PA** (broken line)

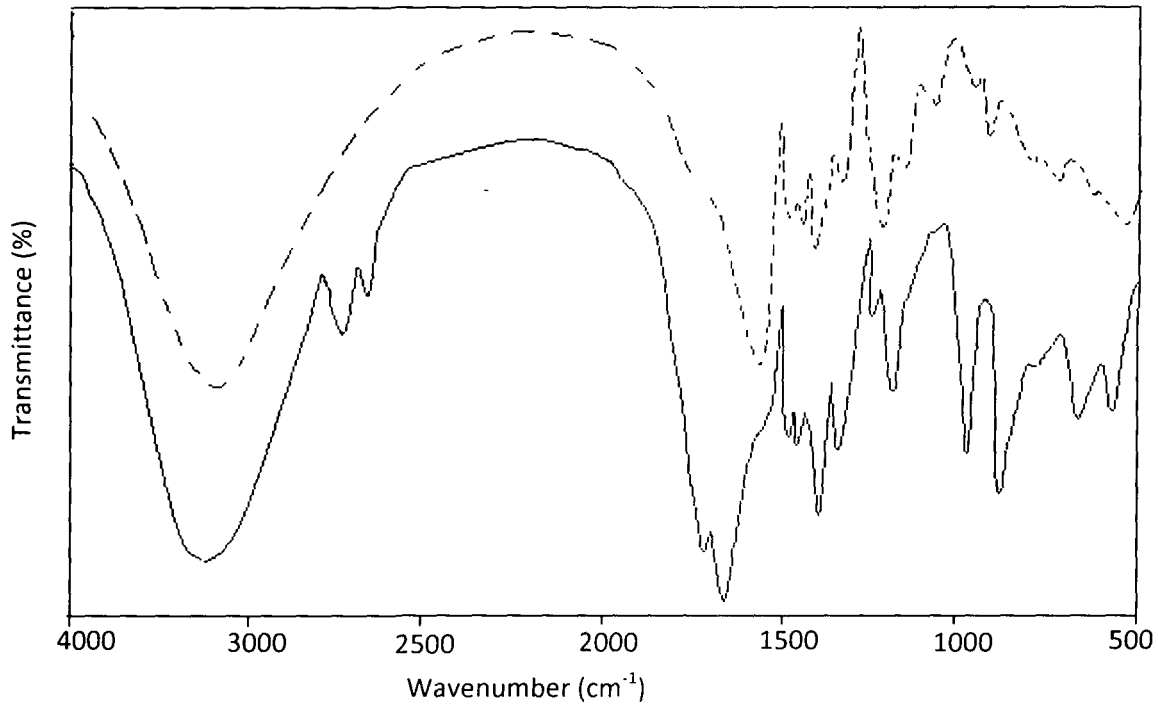

Fig. 3.6 IR spectra of (**PMAV**) [**3.2**] (solid line) and **PMA** (broken line)

be made use of to determine the mode of attachment between the carboxylate group and the metal centre[45,46]. In free sodium poly(acrylate) (NaPA) ν_{asym}(COO) and ν_{sym}(COO) modes are observed at 1565 and 1409 cm^{-1}, respectively ($\Delta\nu$ = 156 cm^{-1}). In the spectra of **PAV[3.1]**, a slight shifting of the ν_{sym}(COO) band to a higher frequency of 1573 cm^{-1} was observed however, the $\Delta\nu$ remained close to that observed for free PA. The observation clearly suggested that the poly(acrylate) chain through its carboxylate group co-ordinate to V(V) in a bidentate bridging fashion. In case of the PMA bound peroxovanadate **PMAV[3.2]**, the bands attributable to ν_{asym}(COO) and ν_{sym}(COO) appeared at 1660 and 1406 cm^{-1} respectively. The distinct shift of the ν_{asym} (COO) to a higher frequency and that of ν_{sym}(COO) to a lower frequency compared to the corresponding free polymeric ligand values of 1540 and 1415 cm^{-1} is typical of monodentate co-ordination of the carboxylate group. Weak bands in the far IR region between 500 and 400 cm^{-1} have been assigned to metal oxygen vibrations. The IR spectral data thus provided clear evidence for the bonding of V-peroxo moiety to PA and PMA in two different fashions. In each of the compounds **PAV[3.1]** and **PMAV[3.2]** the presence of free –COOH groups was evident from the additional band appearing at *ca.*1712 cm^{-1} region. The spectra of the compounds exhibited the characteristic bending $-CH_2$ mode at *ca.*1465 cm^{-1}. Occurrence of lattice water in each of the complexes was apparent from the appearance of strong ν(OH) absorptions displayed at 3500-3400 cm^{-1}.

In the spectra of both the compounds **PSSV[3.3]** and **PSS-co-MV[3.4]**, the band attributable to $\nu_{asym}(SO_3)$ and ν_{sym} (SO_3) appeared in the range of 1185-1190 cm^{-1} and at *ca.* 1128 cm^{-1}, respectively *Fig. 3.7.* The shifting of $\nu_{asym}(SO_3)$ band to lower frequency compared to sulfonate group of the corresponding free polymer value of 1219 cm^{-1} for polymer PSS and 1209 cm^{-1} for polymer PSS-*co*-M provided clear indication of coordination of the sulfonate group to the V(V) centre in each of the compounds[47]. The spectra of the

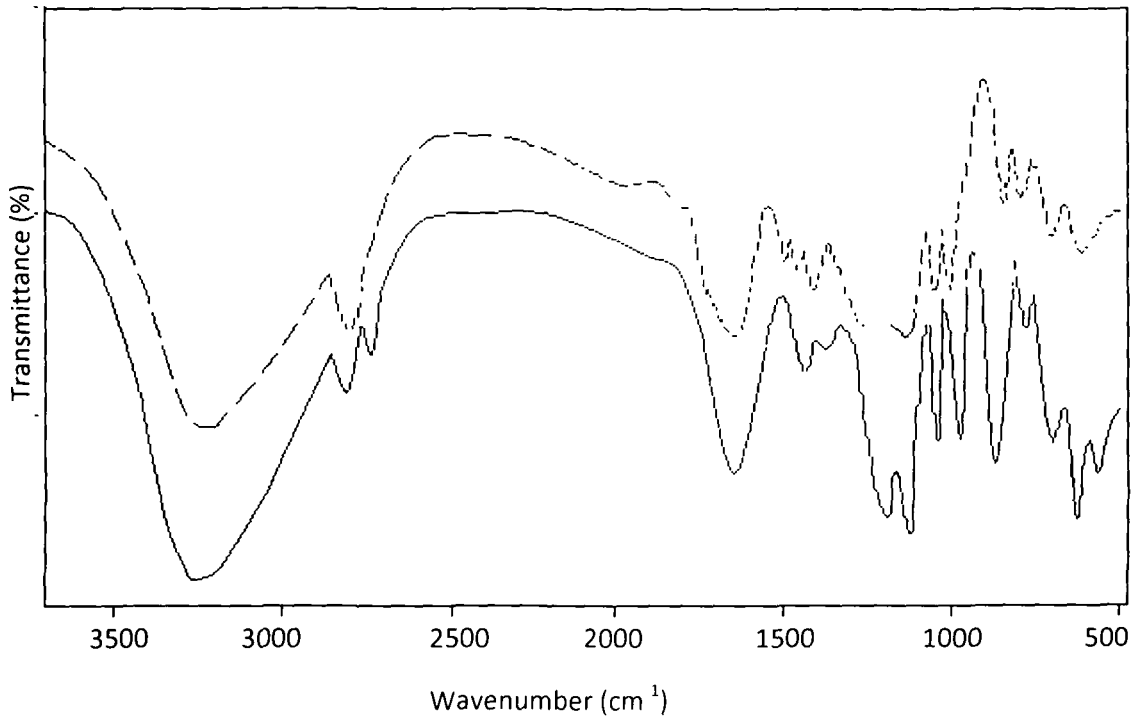

Fig. 3.7 IR spectra of (**PSSV**) [**3.3**] (solid line) and PSS (broken line)

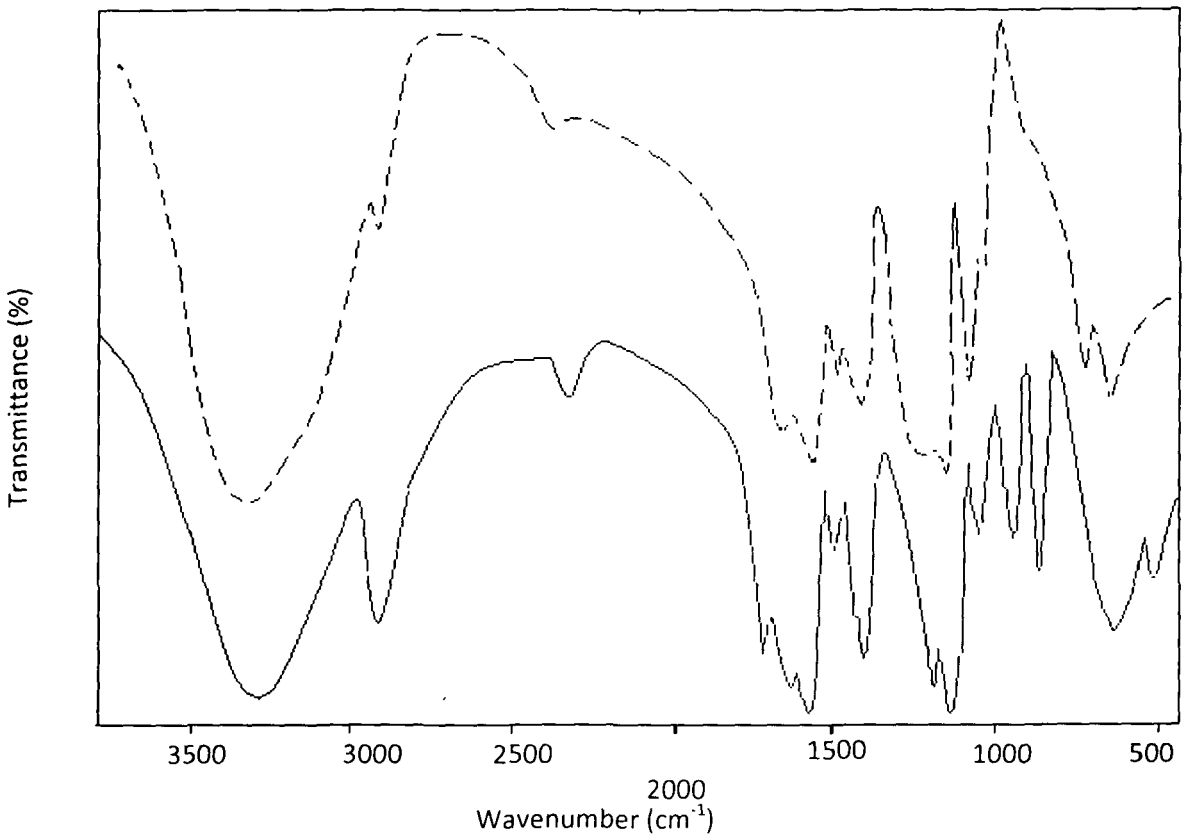

Fig. 3.8 IR spectra of (**PSS-co-MV**) [**3.4**] (solid line) and PSS-co-M(broken line)

compounds exhibited the characteristic absorptions at *ca* 1640 cm^{-1} and 1494 cm^{-1} due to its phenyl group and bending CH_2, respectively. For the compound **PSS-*co*-MV[3.4]** with the maleate functional group on the supporting co-polymer chain, the spectrum displayed bands at 1584 and 1408 cm^{-1} characteristic of the ν_{asvm} and ν_{sym} modes of carboxylate group. Frequency difference between the symmetric and antisymmetric stretches ($\Delta\nu = \nu_{asym} - \nu_{sym}$) of the carboxylate group, is 176 cm^{-1} which is close to that observed in the spectrum of the free polymer (167 cm^{-1}). The observation suggested that the carboxylate group PSSM chain co-ordinate to V(V) in a bidentate bridging fashion. Presence of free –COOH group in the compound **PSS-*co*-MV[3.4]** was evident from the additional band appearing at 1710 cm^{-1}. From the IR spectral data involvement of both sulfonate and carboxylate groups of the co-polymer to in bonding the metal centre was thus evident . Appearance of strong absorptions displayed at 3500-3400 cm^{-1} indicated the presence of lattice water in the complex.

The electronic spectra of the compounds **3.1 - 3.4** in aqueous solution displayed a weak intensity broad band at 320 nm (*Fig 3 9. - 3 12*) which was assigned to peroxo to vanadium (LMCT) transition. The band was observed in the range characteristic of a diperoxovanadate(V) species

On the basis of the above results the proposed structure of polymer anchored pV complex in **PAV[3.1]**, that includes two V atoms co-ordinated to the polymer chain through a bidentate bridged carboxylate group, side-on bound peroxo ligands and terminal V=O, is shown schematically in *Fig 3 13* . For **PMAV**[3.2], a structure of the complex species, incorporating unidentate co-ordination of carboxylate of the polymer to a diperoxovanadate

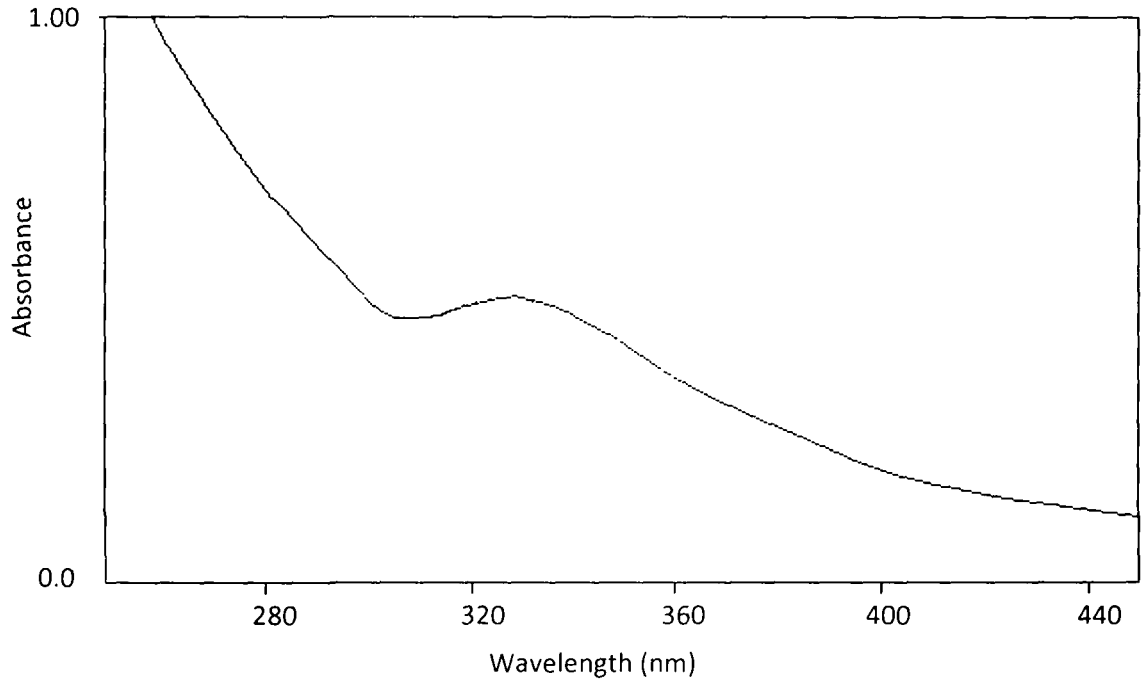

Fig. 3.9 UV spectra of (**PAV**) [**3.1**]

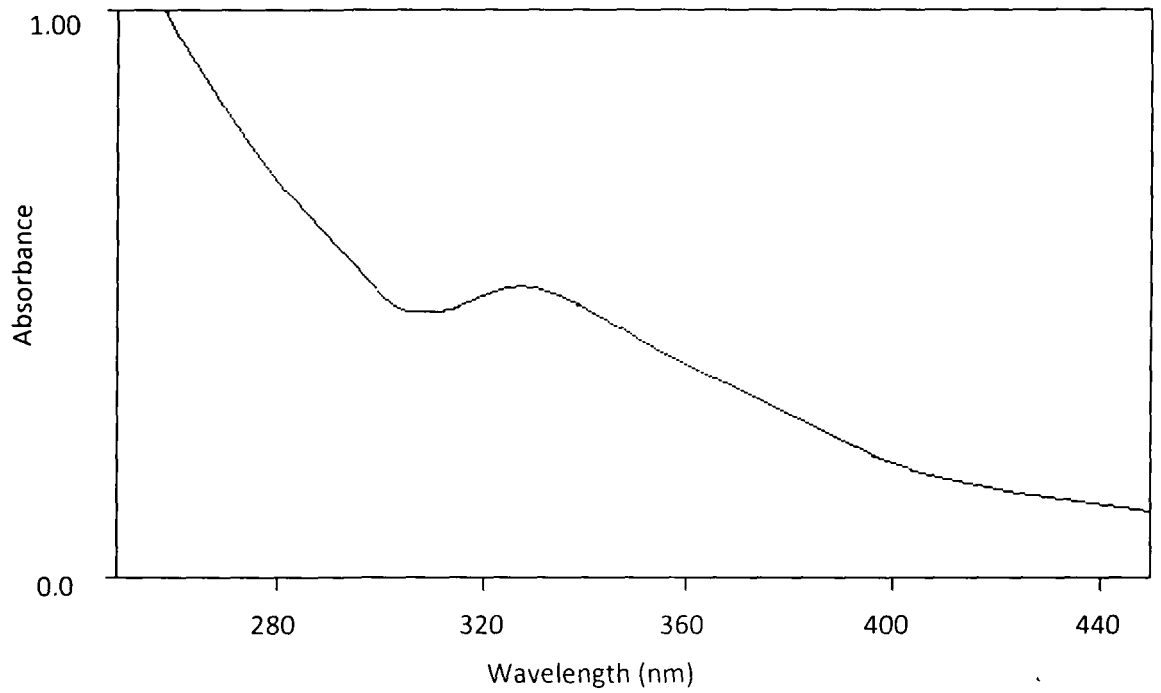

Fig. 3.10 UV spectra of (**PMAV**) [**3.2**]

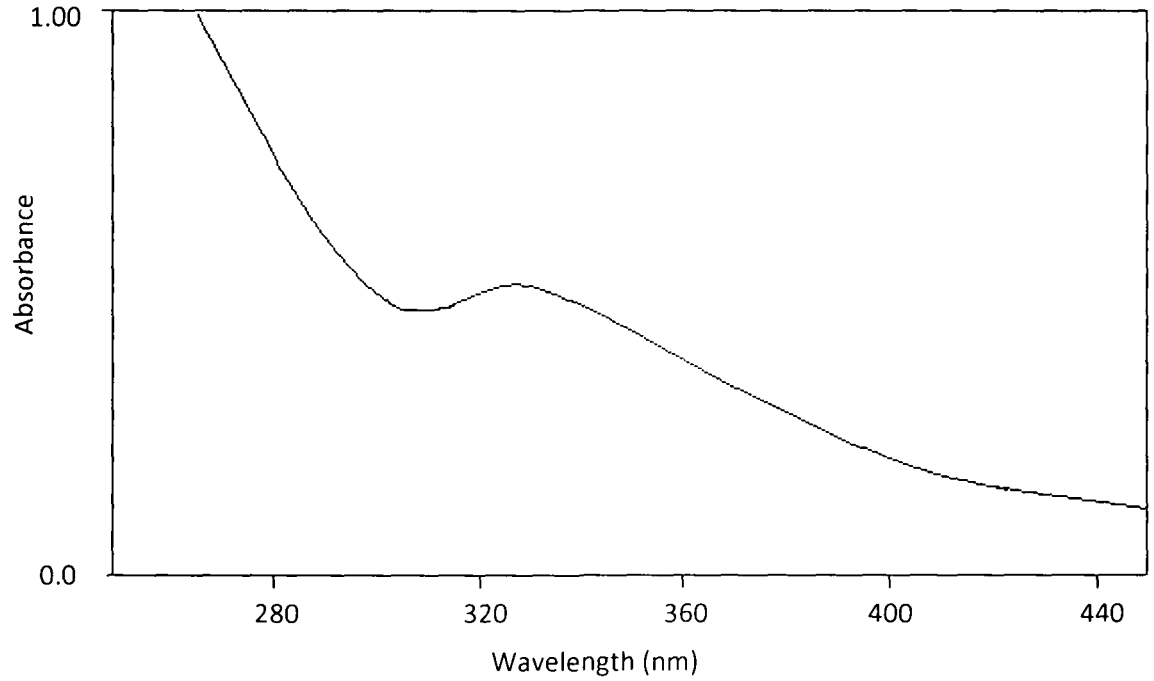

Fig. 3.11 UV spectra of (**PSSV**) [**3.3**]

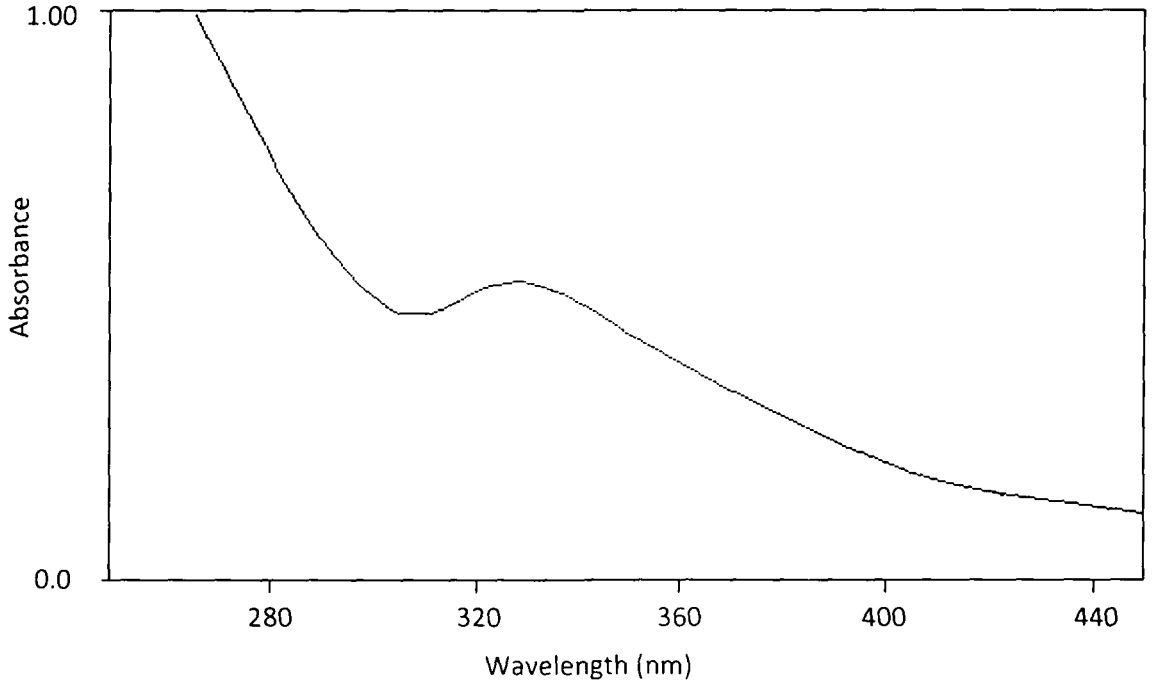

Fig. 3.12 UV spectra of] (**PSS-co-MV**) [**3.4**]

unit has been envisaged (*Fig. 3.14.*). A structure of the type shown in *Fig 3.15*, incorporating unidentate co-ordination of sulfonate group of **PSS** polymer to a diperoxovanadate moiety has been envisaged for the polymer anchored **pV** complex, **PSSV**. The proposed structure for the complex **PSS-*co*-MV** that includes V atoms co-ordinated to the polymer chain via bridged carboxylate of maleate group, unidentate sulfonate group, side on bound peroxo and terminal V=O is shown in *Fig 3.16.*

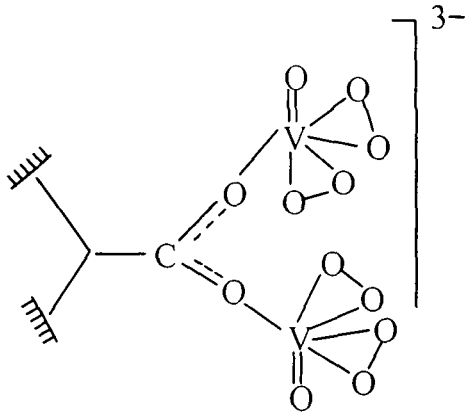

Fig. 3.13. Proposed structures of **PAV [3.1]**

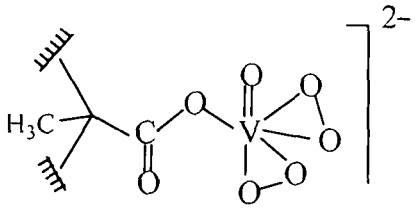

Fig. 3.14. Proposed structures of **PMAV [3.2]**

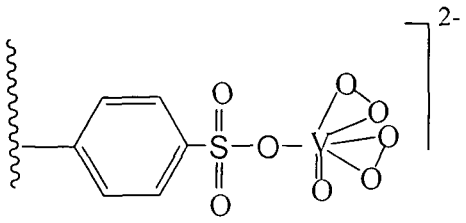

Fig. 3.15 Proposed structures of (**PSSV**) [**3.3**]

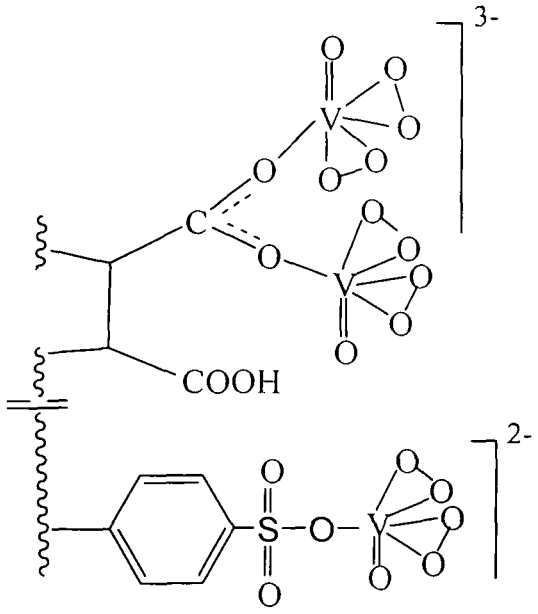

Fig. 3.16 Proposed structures (**PSS-co-MV**) [**3.4**]

The triglycine containing compound, **pV1[3.5]** exhibited characteristic spectral pattern in the IR region indicating the presence of co-ordinated oxo, peroxo, co-ordinated peptide and lattice water in it. The existence of coordinated peptide in the complex **pV1[3.5]** was evident from IR spectrum, which showed characteristic shifts of heteroligand bands that occurred upon coordination compared to the spectra of the free ligand. The IR spectra of triglycine and a limited number of metal complexes with coordinated triglycine were

investigated previously[48-50]. Assignments of the peaks observed were made based on the data available. A distinct broad band was observed for the compounds **pV1[3.5]** in the range of 1660-1650 cm^{-1} representing the ν(C=O) of coordinated tripeptide ligand. The band was shifted to lower frequency with some broadening, as compared to free triglycine (1684 cm^{-1}) clearly indicating participation of the amide carbonyl group in metal bonding. Co-ordination through N-atom of the amide group was unlikely since it is known to cause much larger decrease in peptide carbonyl stretching frequency than observed in the present case[51,52]. The broadening of the band was probably due to the presence of both co-ordinated as well as free amide

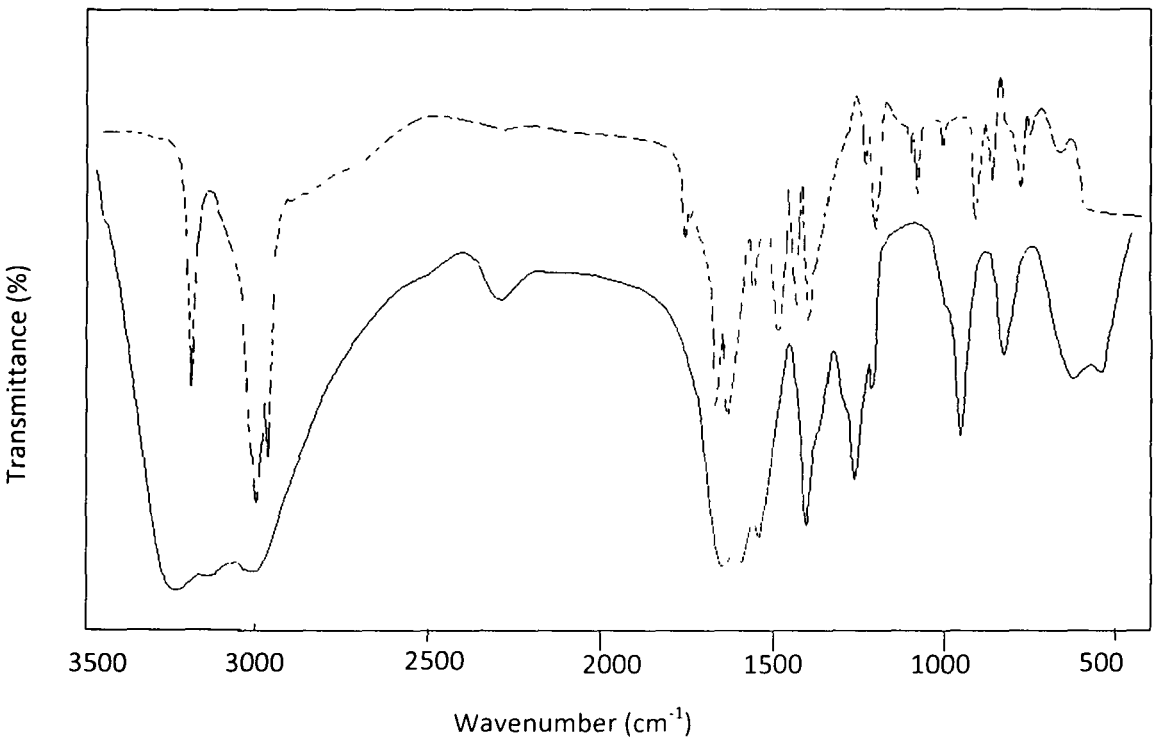

Fig. 3.17 IR spectra of $Na[VO(O_2)_2(triglycine)].3H_2O$ (**PV1**)[**3.5**] (solid line) and triglycine (broken line)

groups in the compounds. The absorptions attributable to antisymmetric and symmetric stretching frequencies of carboxylate group appeared at *ca.* 1598-1605 and 1385-1390 cm^{-1}, respectively. The shifting of the ν_s(COO) band to a lower frequency from the 1402 cm^{-1} of free ligand value is typical of unidentate co-ordination of carboxylate group[51]. The spectra showed N-H stretching bands of coordinated peptide residue at *ca* 3300-3100 cm^{-1} region and $\delta(NH_2)$ at *ca.* 1550 cm^{-1} as expected from the $^{+}NH_3$ group. The rocking modes of $^{+}NH_3$ occurred at *ca.*1130 and *ca.*1042 cm^{-1}. The presence of water in the complexes was evident from the broad absorption at 3500-3400 cm^{-1}, due to ν(OH).

Above results are consistent with structure of the complex shown in *Fig. 3.18*. The tripeptide ligand occurring as zwitterion in the complex, co-ordinate to vandaium(V) through carboxylate group. Coordination of one of the carbonyl (amide) groups of the peptide chain probably completes the hepta coordination of the respective metal centre. The second amide group in the peptide side chain is not shown in the structure for simplicity.

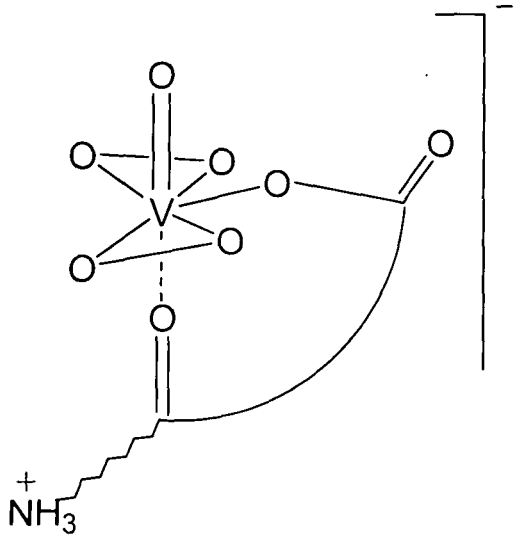

Fig. 3.18 Proposed structure of the **(pV1) [3.5]**

Electronic spectra of the compound **pV1[3.5]** like the polymer anchored complexes exhibited a weak intensity broad band at 320-330 nm (*Fig. 3.19*) in aqueous solution which is attributable to LMCT transitions originating from co-ordinated peroxide and is typical of diperoxo derivatives of vanadium.

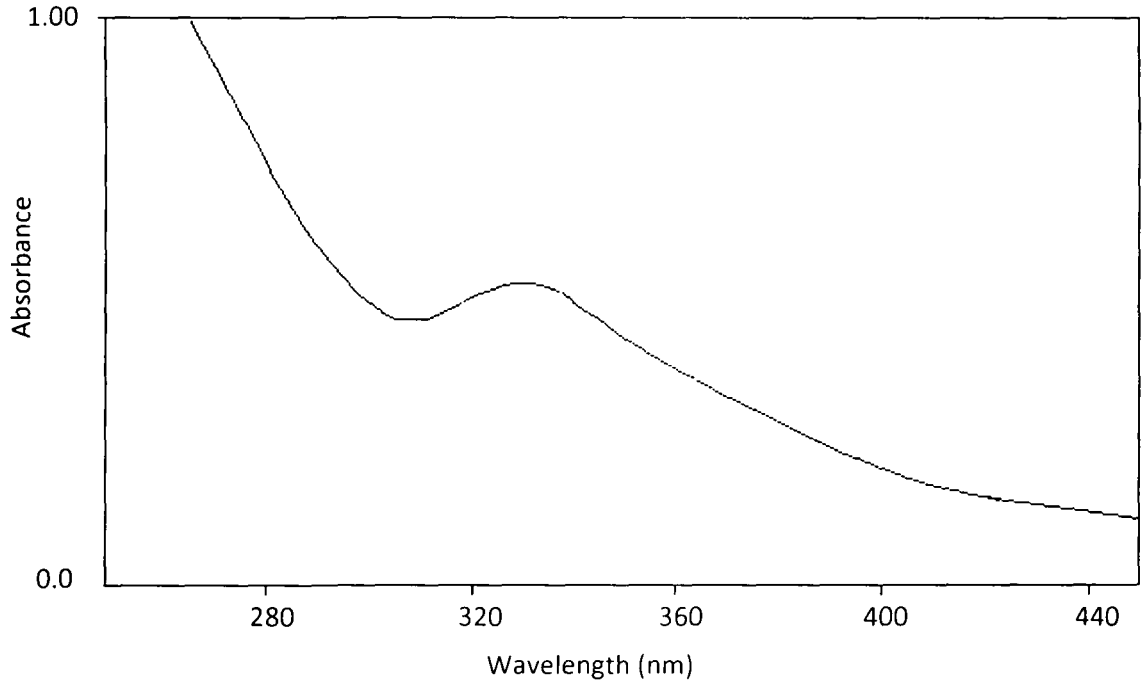

Fig. 3.19 UV spectra of $Na[VO(O_2)_2(triglycine)].3H_2O$ (**PV1**)[**3.5**]

3.3.1.5 Thermal analysis

The thermograms of the compounds **3.1-3.4** are presented in *Fig 3.20 - 3.23*. Thermogravimetric analysis data (Table 3.3) indicated that after the initial dehydration, the compounds undergo multistage decomposition. The first stage of decomposition occurring between 40-110 ^{0}C with the liberation of molecules of water of crystallization from the complexes with a corresponding weight loss of 12.5% (**PAV[3.1]**) and 16.0% (**PMAV[3.2]**), 10.2 % (**PSSV[3.3]**) and 9.8 % (**PSS-co-MV[3.4]**). The next decomposition stage is in the

temperature region of 90-250 ^{0}C for **PAV[3.1]**, 90-180 ^{0}C for **PMAV[3.2],** 110-250 for **PSSV[3.3]** and 110-200 ^{0}C for **PSS-*co*-MV[3.4]** with a corresponding weight loss of 13.0%, 10.0%, 7.5 % and 10.9 % respectively attributable to complete loss co-ordinated peroxo groups from the complexes. Absence of peroxide in the decomposition product, isolated at this stage, was confirmed from the IR spectral analysis. The loss of peroxide is seen to be followed by a three stage decomposition occurring in the broad temperature range of 290-800 ^{0}C for **PAV[3.1]** and 310-600 ^{0}C for **PMAV[3.2]** respectively which may be attributed to decarboxylation involving free as well as coordinated carboxylate functionals accompanied by rupturing of the polymers. For **PSSV[3.3]** the loss of peroxide is seen to be followed by a two stage decomposition in the range 420-600 ^{0}C due to the loss of sulfonate group and rupturing of polymers whereas three stage decomposition decomposition occurs in the broad temperature range of 290-650 ^{0}C for **PSS-*co*-MV[3.4]** which may be attributed to decarboxylation involving free as well as coordinated carboxylate and sulfonate functionals accompanied by break-up of the polymer matrix. Further evidence regarding decarboxylation and desulfoantion of the polymers was obtained from the IR spectra recorded after heating the compounds separately up to the final decomposition temperature which showed complete disappearance of the strong peaks originating from ν_{asym}(COO) ,ν_{sym}(COO), ν_{asym}(SO_3) and ν_{sym}(SO_3) of the spectra of the title compounds. The total weight loss which occurred during the course of the overall decomposition process on heating the compounds up to a final temperature 800 ^{0}C was recorded to be 64.0% for **PAV[3.1]** and 56.0% for **PMAV[3.2]** due to the complete loss of the components viz. lattice water, coordinated peroxide and polymeric functionals. For the compound **3.3** and **3.4** the total weight loss which occurred during the course of the overall decomposition process on heating the compounds

Table 3.3

Thermogravimetric data of peroxovanadium complexes **3.1-3.4**

Compound	Temperature range (^{0}C)	Observed weight loss (%)	Final residue (%)
1. PAV [3.1]	40-90	12.5	
	90-250	13.0	
	290-800	38.5	36.0
2. PMAV[3.2]	40-90	16.0	
	90-180	10.0	
	310-600	30.0	44.0
3. PSSV[3.3]	40-110	10.2	
	420-600	7.5	
	420-600	35.3	47.0
4. PSS-*co*-MV[3.4]	40-110	9.8	
	110-200	10.9	
	290-650	32.0	47.3

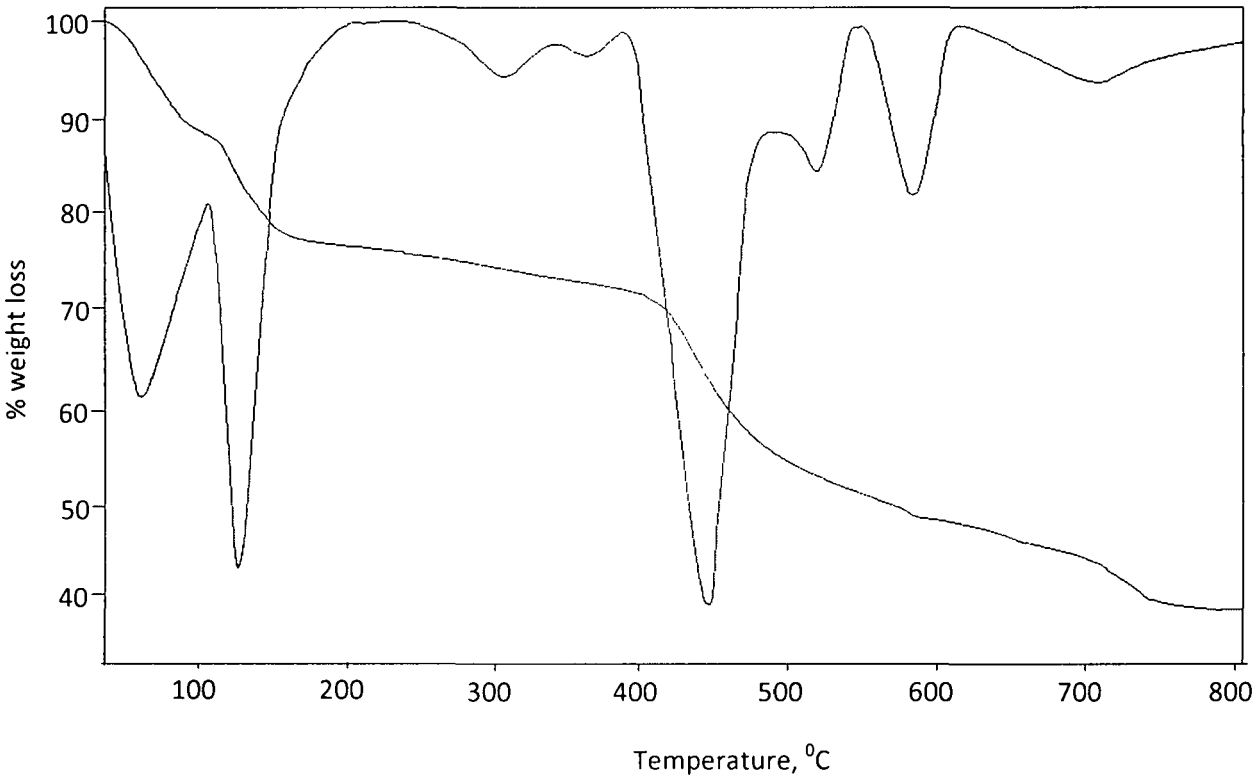

Fig. 3.20 TG-DTG of (**PAV**) [**3.1**]

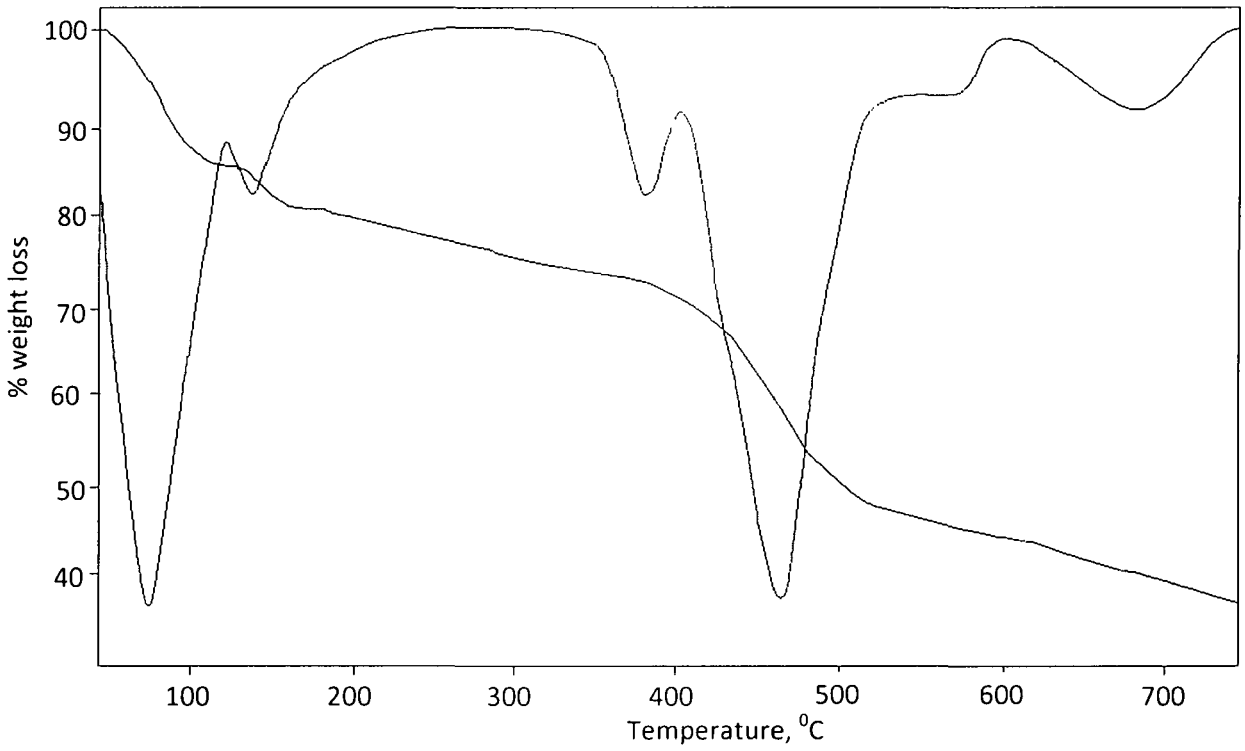

Fig. 3.21 TG-DTG of (**PMAV**), [**3.2**]

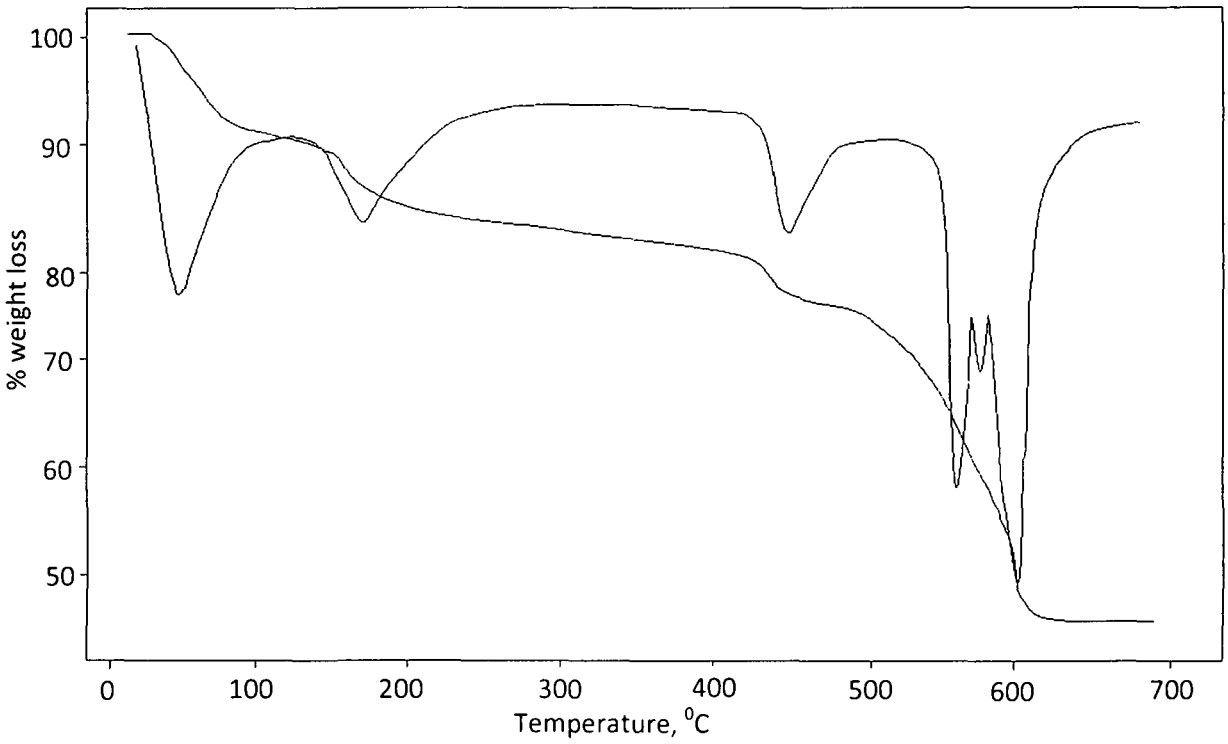

Fig. 3.22 TG-DTG of (**PSSV**) [**3.3**]

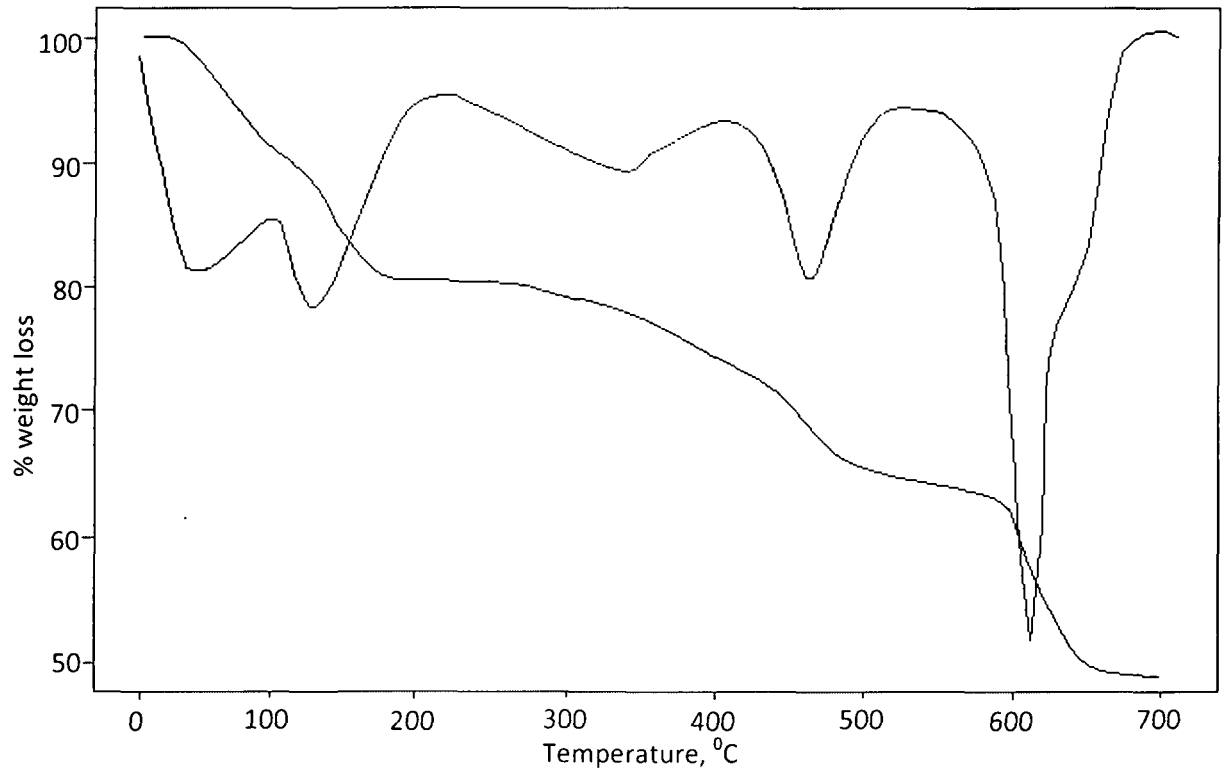

Fig. 3.23 TG-DTG of (**PSS-co-MV**) [**3.4**]

up to a final temperature 650 ^{0}C was recorded to be 53.0 % and 52.7 % respectively, due to the complete loss of the components viz. lattice water, coordinated peroxide and polymeric functionals. The IR spectra of the residue remaining at this stage showed the presence of oxovanadium species. Thermogravimetric analysis data of the compounds thus provided further evidence in support of the composition and formula assigned to the compounds.

The thermogravimetric analysis for **pV1[3.5]** shows that the compound undergo multistage thermal decomposition in 40-500 ^{o}C (*Fig.3.24.*). The first stage of decomposition for both the compounds occurs at 40-100 ^{0}C with the liberation of the water molecules from the complex. The observed weight loss of 12.8% is in good agreement with the corresponding calculated values of 13.6% for the loss of three molecules of water of crystallization from the complex. After the dehydration, the compounds undergo continuous degradation with loss of peroxide and trigycine ligand up to final decomposition temperature of 500 ^{0}C. The total weight loss which occurred during the course of the overall decomposition process was recorded to be 76.8%, which agree well with the theoretically calculated values of 77.3 % for the complete loss of the components viz. water molecule, co-ordinated peroxide, and the triglycine ligand. The remaining dark brown sticky residue from **pV1[3.5]** was found to be a hydrated oxovanadate species as indicated by the IR spectrum which displayed the characteristic ν(V=O) was devoid of bands attributable to peroxo and the tripeptide ligand of the original compound. Thermogravimetric analysis data of the compound thus provided further evidence in support of the composition and formula assigned.

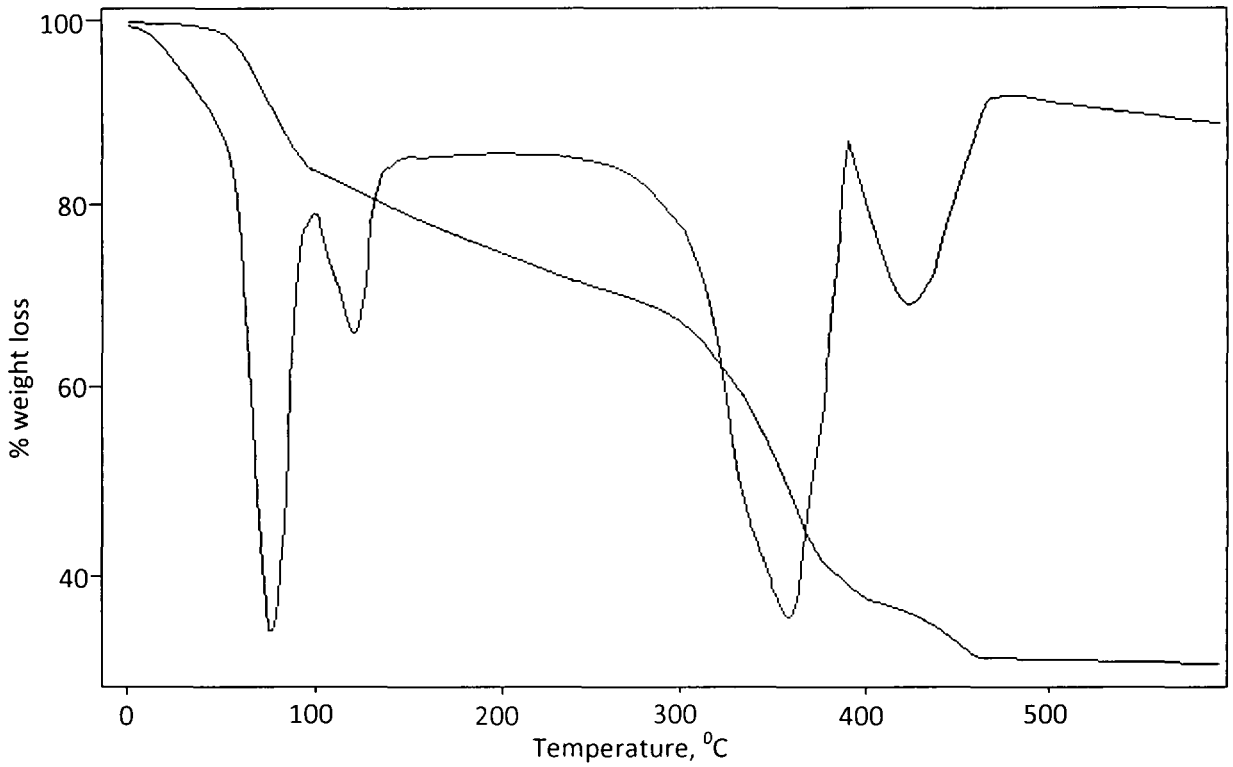

Fig. 3.24 TG-DTG of (**PV1**)[**3.5**]

3.3.2 Stability of the complexes in solution

The investigations on the stability of the compounds in an aqueous solution of pH *ca.* 6, which is the natural pH attained by the solution of the polymer bound compounds **3.1** – **3.4** as well as **3.5** in water has been studied by estimating their peroxide content and absorbance at 320 nm region in the electronic spectra at specified time interval for any possible change. The investigation revealed that their peroxide content and position and intensity of their electronic spectral bands remained unaltered for over a period of 24 h. (*Fig.*) We further examined and ascertained their stability in solutions of pH values ranging from 3.6 to 8.0 (data not shown). Furthermore, the molar conductance of anionic **pV1[3.5]**, which revealed 1:1 electrolytic nature of the compound, remained unchanged during the period of

investigation. The above result demonstrates that the complex species retain their structural identity in solution and clearly attest to the hydrolytic stability of the complexes.

3.4 DISCUSSION

One of the advantages of using a soluble polymeric ligand for the synthesis of polymer-bound metal complexes is the possibility of adopting synthetic procedures used for preparing their low molecular weight analogues. The methodology for the successful synthesis of the polymer anchored peroxovanadates **3.1-3.4** was based on the reaction of V_2O_5 with H_2O_2 and Na salt of the respective polymeric ligand in an aqueous medium. Chelation of a metal ion by a polymeric ligand such as polycarboxylate, as well as composition of peroxo-vanadium species have been known to be sensitive to pH of the reaction mediu[45-48],[53-56] . In a solution of vanadate and excess H_2O_2 at a pH>5, formation of diperoxovanadate (DPV) species is favoured[57]. Depending on the pH of the reaction solution and the nature of the metal a carboxylate group can act as a monodentate, bidentate chelating or bidentate bridging ligand[51]. In the present study, the strategically maintained pH of *ca.* 6 was found to be optimum for the formation of the diperoxovanadate moieties and their co-ordination to the carboxylate group in **PAV[3.1]** and **PMAV[3.2]**, sulfonate and maleate in **PSS-co-MV[3.4]** and **PSSV[3.3]**, respectively leading to the desired synthesis of the compounds. It is quite intriguing to note that the carboxylate functional group present in the two closely related polymer matrices, poly(acrylate) and poly(methacrylate) binds the pV moieties in two different co-ordination modes leading to the formation of two structural forms of peroxovanadates viz., a dimeric tetraperoxovanadate in **PAV[3.1]** and monomeric diperoxovanadate in **PMAV[3.2]**. This difference in co-ordination pattern may be attributed to the presence of $-CH_3$ groups attached to the polymer backbone in PMA which being

relatively bulkier probably prevents the formation of a dinuclear pV species through carboxylate bridge. Similarly the binding of pV to polymer chain in its dinuclear form via bridging co-ordination of carboxylate group, as has been observed in case of the compound **PSS-*co*-MV[3.4],** is not unprecedented.

From our investigation on the stability of the compounds in solution it is confirmed that these compounds remain intact for a reasonable period of time, in solution of neutral as well as acidic pH. The enhanced stability of the macromolecular peroxovandate may be attributed to the additional stability imparted by polymeric support.

In summary, the present investigation has established that it is possible to isolate well defined and stable peroxovanadium species anchored to suitable macro ligands under appropriate experimental conditions. These are probably the first known examples of peroxovanadate complexes where water soluble polymers or a co-polymer are used as supports. Synthetic route to a new tripeptide containing pV has also been achieved. A noteworthy finding of the present investigation, which may also be of biochemical importance is that the compounds remain stable in solution of a wide range of pH particularly at acidic pH. Results of investigation on the interaction of the title compounds with the enzyme catalase and their effect on the alkaline phosphatase activity, antibacterial properties as well as their redox properties are presented in Chapters **4, 5** and **6** of the thesis.

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CHAPTER 4

Effect of polymer bound and free peroxovanadate compounds on activity of alkaline phosphatase and their interaction with catalase

4.1 INTRODUCTION

Alkaline phosphatase (ALP) is a membrane-bound zinc metalloenzyme widely distributed in nature, from bacteria to plants to human[1]. While specific chemical reactions catalyzed by these enzymes are well characterized, their role in cellular metabolism and regulation is less clearly defined[2]. The enzyme is characterized by its ability to catalyze the hydrolysis of phosphomonoesters. Phosphotransferase activity and protein phosphatase activity are some of the other probable functions assigned to the enzyme. ALP has a broad susbstrate specificity[3], and its pH optimum is usually around 9 [3,4]. Alkaline phosphatase assays, extensively used in immunoassays, are of clinical importance because increased or decreased levels are usually indicative of disease[3,4]

Vanadium and its compounds including peroxovanadates have strong influences, inhibiting the function of a large number of enzymes and promoting the function of others[5-10] There has been a growing awareness on the importance of enzyme inhibition as a mode of action for inorganic drugs in recent years[11] and is a thriving area of current research. It is now well established that vanadate and peroxovanadate are capable of inhibiting the hydrolysis of phosphoproteins and

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Results described in this chapter have been accepted for publication in:
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exhibit insulin-like properties[8-10,12]. The exact mechanism by which peroxovanadate mimic the action of insulin or inhibit the enzyme function is yet to be fully established[9-12]. Correlation has been found between abilities of vanadates and pV to inhibit protein phosphatases and to promote activation of insulin receptor, and their *in vivo* insulin mimetic activities[8-10]. In fact, ability of vanadates to inhibit phosphohydrolase enzymes is recognized as key to understanding bioactivity of vanadium[10] In order to gain an insight into the role of vanadium in bioprocesses a variety of synthetic peroxovanadium compounds with different ancillary ligands have been studied as biomimetic models and have also been tested for their pharmacological potential[12-14] . However, the currently used therapeutic peroxo vanadium compounds appear to dissociate under physiological conditions[7]. It is notable in this context that a set of peroxo compounds of V(V) and W(VI) containing amino acids and dipeptides as co-ligands, synthesised recently in our laboratory, were not only stable in solution of a wide range of pH values, but also induced strong inhibitory effect on the activity of rabbit intestine ALP. Inhibitor potency of these compounds appeared to be sensitive to the ligand employed[15-17].

Keeping in view the afore mentioned observations and in continuation of our work on peroxovanadates described in Chapter **3**, which afforded stable pV complexes anchored to soluble polymeric matrices, we deemed it worthwhile to examine some of the biochemically important features of the newly synthesised macro complexes such as their possible effect on ALP and their interaction with the enzyme catalase. One of the specific interests was to explore whether binding of low molecular weight pV species to macromolecular ligands would alter their affinity as enzyme inhibitor. In order to address this aspect, we have decided to undertake a comparative study of the polymer-bound as well as free pV complexes with respect

to their effect on ALP, using a set monomeric heteroligand diperoxovanadium compounds with appropriate characteristics of solubility and stability, including the newly synthesized tripeptide peroxovanadate (pV1) . It has been expected that information gained from such studies would be important in understanding the influence of ligand environment of the complexes on their properties tested. A survey of literature shows that no peroxometal complex anchored to polymer has so far been studied for its effect on alkaline phosphatase activity.

It was also considered imperative to examine the possible fate of the peroxo compounds in presence of catalase, since the primary objective of our work has been to generate information on some of the biologically relevant properties of the complexes. Catalase is the ubiquitous enzyme responsible for breakdown of H_2O_2 formed during oxidative processes in the intercellular peroxisomes. There are many heterogenized enzymetic systems, obtained by immobilization of catalase on synthetic polymers[18].

Presented in this chapter are the findings of our investigation on the kinetics of inhibition of rabbit intestine ALP by the polymer anchored peroxovandaium compounds viz., $[V_2O_2(O_2)_4$(carboxylate)]-PA(**PAV**) **[3.1]**, $[VO(O_2)_2$(carboxylate)]-PMA(**PMAV**)(**3.2**), $[VO(O_2)_2$(sulfonate)]-PSS (**PSSV**) **[3.3]**, $[V_2O_2(O_2)_4$(carboxylate)$VO(O_2)_2$(sulfonate)]-P(SS-*co*-M) (**PSS-*co*-MV**) **[3.4]** as well as, monomeric heteroligand peroxovandates of the type $Na[VO(O_2)_2$(triglycine)].$3H_2O$ (**pV1**)**[3.5]**, $Na[VO(O_2)$(gly-gly)$(H_2O)].H_2O$ (**pV2**) **[4.1]**. $Na[VO(O_2)_2$(gln)].H_2O (**pV3**)**[4.2]**, $Na[VO(O_2)_2$(asn)].H_2O (**pV4**)**[4.3]**, Results of studies on the interaction of the polymeric complexes with the enzyme catalase are also incorporated herein

4.2 EXPERIMENTAL SECTION

4.2.1 Measurement of alkaline phosphatase activity

Phosphatase activity was assayed spectrophotometrically by using p-nitrophenyl phosphate (p-NPP) as a substrate. The continuous production of p-nitrophenol (p-NP) was determined at 30 ^{0}C by measuring absorbance at 405 nm in a reaction mixture containing ALP from rabbit intestine (3.3 μg protein/ ml), p-NPP (2-5 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0). The initial reaction rates were obtained by starting the reaction by adding ALP to the reaction solution, which was pre-incubated for 5 min. The initial reaction rate of p-NPP hydrolysis in the absence of the inhibitors, V_0 was determined which was used as control. The effects of pV and other inhibitors were assessed by adding different concentrations (2-50 μg/ml) of each species in the ALP assay. The IC_{50} values were graphically determined as the half-maximal inhibitory concentration of the inhibitor species giving 50% inhibition. All the assays were performed in triplicate. The data in figures are presented as the means ± SE from three separate experiments.

4.2.2 Determination of kinetic parameters

The kinetic parameters V_{max} and K_m of an enzyme catalysed reaction were determined using Lineweaver –Burk plot following rearrangement of the Michaelis Menten equation since Lineweaver Burk plot is the most popular linear form of the Michaelis Menten equation containing a plot of 1/Vo (Y axis) vs 1/S (X axis)

$$\frac{1}{V} = \left\{ \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}} \right\}$$

The parameter V_{max} is the maximal velocity and K_m is the Michaelis constant, its value being equivalent to the substrate concentration at which velocity is equal to half of $V_{max}/2$. V_{max} and K_m can be obtained from the intercept and slope of the lineweaver Burk plot containing a plot of 1/Vo (Y axis) vs 1/S (X axis) X intercept= 1/Km, Y intercept= $1/V_{max}$.

Different types of inhibition give the different plot patterns. For instance with a Lineweaver Burk plot, the lines will converge on the Y axis when there is competitive inhibition, but the lines will be parallel when the inhibition is uncompetitive. For noncompetitve inhibition, the lines will converge either on the X axis (simple noncompetitive inhibition) or above /below the X axis (mixed inhibition).

Noncompetitive inhibition occurs when an inhibitor binds to both the enzyme and the enzyme substrate complex. Competitive inhibition occurs when an inhibitor competes with the substrate for binding at the enzymes active site. Uncompetitive inhibition occurs when an inhibitor binds to the enzyme – substrate complex but not to free enzyme.

In the present case the expression for velocity of the reaction is given by

$$V = \left\{ \frac{V_{max} \times [S]}{K_m\left(\frac{1+[I]}{K_i}\right) + [S]\left(\frac{1+[I]}{K_{ii}}\right)} \right\}$$

where v is the velocity, [S] is the pNPP concentration and [I] is the inhibitor concentration, K_i is the inhibitory constant for the competitive part and K_{ii} is the inhibitory constant for the noncompetitive part. The enzyme inhibitor and enzyme substrate inhibitor constant were calculated from secondary plots of initial rate data by

linear regression analysis. The slopes obtained from Lineweaver plots were replotted against inhibitor concentration to obtain K_I values from the x-intercepts of these replots. The intercepts obtained from Lineweaver plots were replotted against inhibitor concentration to obtain K_{II} values from the x-intercepts of these replots.

4.2.3 Effect of catalase on the complexes

The effect of catalase on complexes was studied by estimating the peroxide content of the compounds in a solution containing catalase at specified time intervals. The test solution contained phosphate buffer (50 mM, pH 7.0) and catalase (40 μg/ml). The volume of the reaction solution was kept at 25 ml. The solution was incubated at 30 ^{0}C. The compound was then added to the test solution and aliquots of 5 ml were pipetted out and titrated for peroxide content after stopping the reaction by adding it to cold sulfuric acid (0.7 M, 100 ml) at time 5, 10, 30, 60, 90 and 120 min of starting the reaction.

4.3 RESULTS AND INTERPRETATION

4.3.1 Effect of catalase on the macromolecular peroxovandaium compounds

Catalase is an effective catalyst for H_2O_2 decomposition to H_2O and O_2. Addition of catalase to phosphate buffered solution of H_2O_2 released a half-equivalent (molecular basis) of oxygen, as expected from disproportionation reaction, which will be completed within 2 min. On incubation with catalase, each of the polymeric compounds **3.1- 3.4** as well as newly synthesized monomeric triglycine

containing peroxovanadate **3.5**, which were otherwise ascertained to be stable in solution of a wide range of pH values, were found to be degraded slowly with the loss of peroxide.

Total peroxide loss from the triglycine peroxovanadate solution of 0.1 mM concentration tested was recorded to be *ca.* 0.2 mM indicating a ratio of 1:2 for peroxide: peroxovanadate compounds which is consistant with the estimated peroxide content of the compounds. The extent and initial rates of degradation of the compound (Table 4.1) under the effect of catalase action was found to be comparable to that of diperoxo compounds of vanadium synthesized and examined previously by us [15,17], indicating their similarity with respect to number of peroxide and probably the pattern of co-ordination to the respective metal centre

The effect of catalase on the macro complex 3.1 – 3.4 is shown in *Fig. 4.1* and *Fig. 4.2,* tested with compounds **3.1** and **3.3** as representatives. Under the effect of catalase action were found to vary within the range of 3.8-5.8 μM/min, (Table 4.1) respectively which are approximately 2-3 fold lower than that observed for free diperoxovanadate (DPV) under similar reaction condition.

Table 4.1. *Catalase dependent oxygen release from pV compounds*

Sl. No. Compounds	Conc	peroxide content	Loss of peroxide
	(mg/ml)	mM	μM /min.
1.$[V_2O_2(O_2)_4$(carboxylate)]-PA(**PAV**) (**3.1**)	0.11	0.4	5.8
2.$[VO(O_2)_2$(carboxylate)]-PMA(**PMAV**) (**3.2**)	0.14	0.4	5.6
3.$[VO(O_2)_2$(sulfonate)]-PSS **PSSV** (**3.3**),	0.23	0.6	3.8
4.$[V_2O_2(O_2)_4$(carboxylate)$VO(O_2)_2$(sulfonate)]-P(SS-*co*-M) **PSS-*co*-MV** (**3.4**)	0.19	0.6	4.0
5.$Na[VO(O_2)_2$(triglycine)].$3H_2O$ (**pV1**)(**3.5**)		0.2	17.5

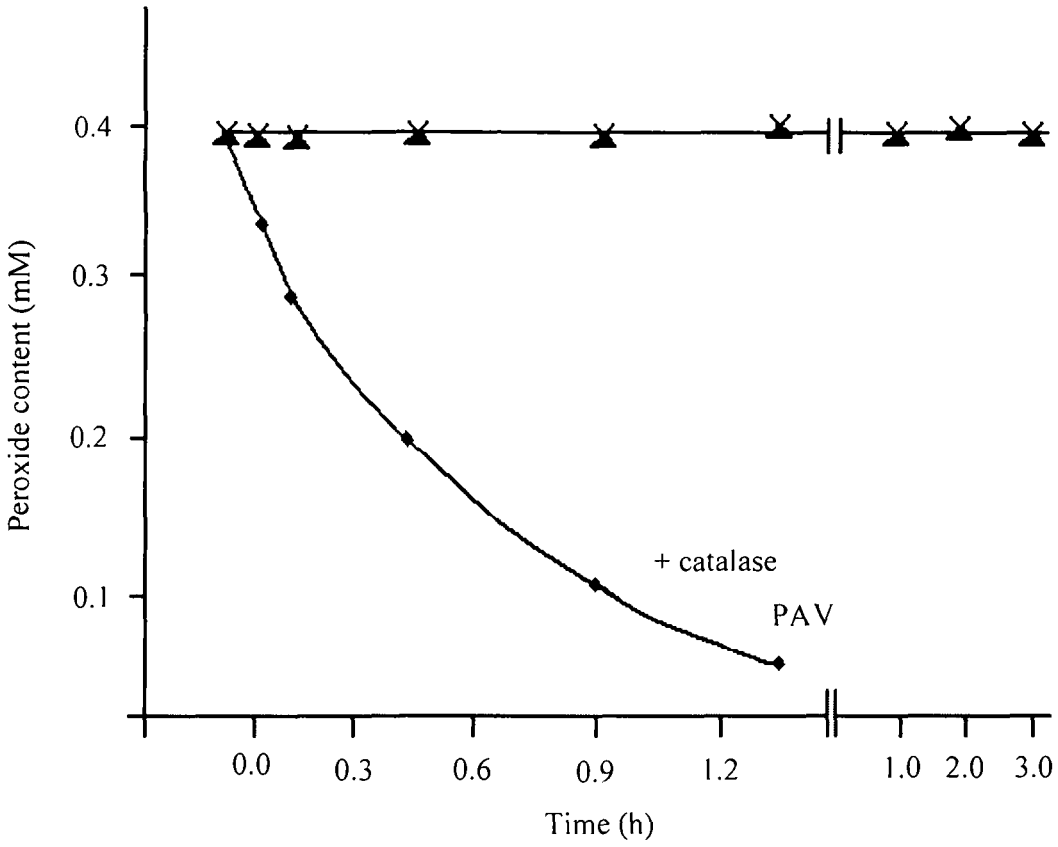

Fig. 4.1. Stability of compound PAV at different pH values, effect of catalase. ×: Compound solution in distilled water (0.11 mg/ml), pH of the solution 6.0. ▲: Solution of complexes in phosphate buffer (pH 7.0) ♦: Effect of catalase. The test solution contained phosphate buffer (50 mM, pH 7.0) and the catalase (40 μg /ml) which was incubated at 30 ^{0}C for 5 min. Compounds (0.11 mg/ml) were then added to the reaction solution and aliquots were drawn at indicated time points and loss in peroxide content was determined.

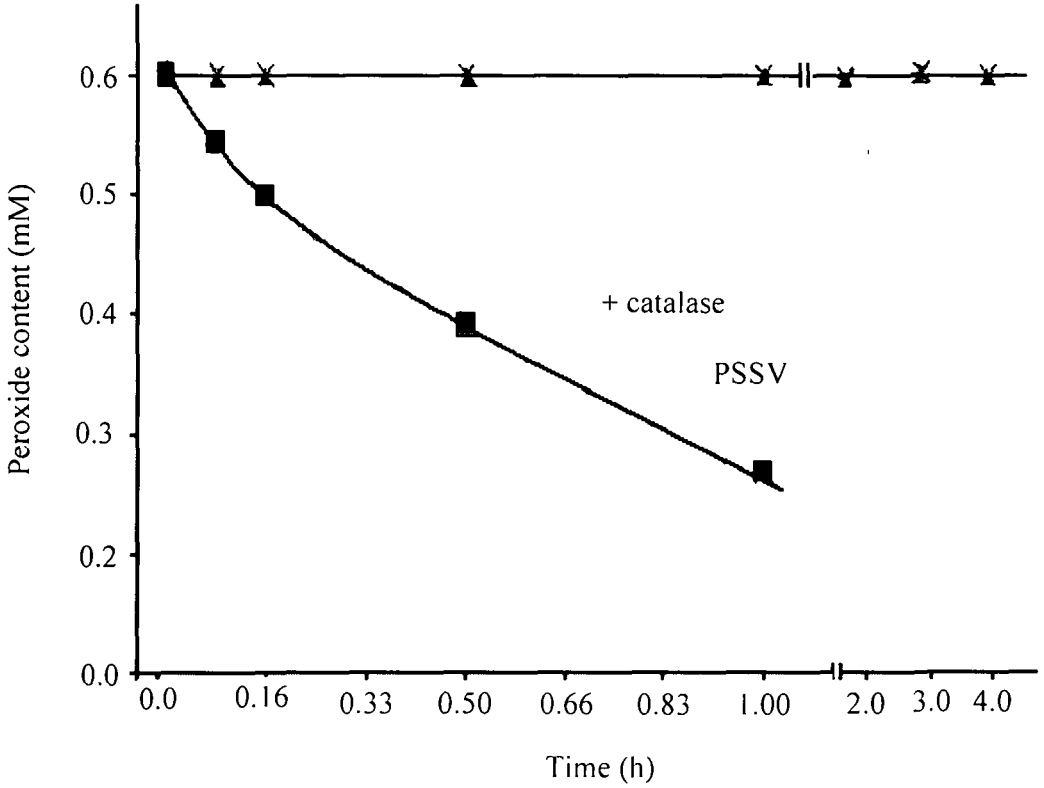

Fig. 4.2. Stability of compound **PSSV** at different pH values, effect of catalase. ×: Compound solution in distilled water (0.23 mg/ml), pH of the solution 6.0. ▲: Solution of complexes in phosphate buffer (pH 7.0) ■: Effect of catalase. The test solution contained phosphate buffer (50 mM, pH 7.0) and the catalase (40 μg /ml) which was incubated at 30 ^{0}C for 5 min. Compounds (0.23 mg/ml) were then added to the reaction solution and aliquots were drawn at indicated time points and loss in peroxide content was determined.

4.3.2 Effect of peroxovanadium compounds on alkaline phosphatase activity

The effect of different concentrations of pV complexes bound to soluble polymeric matrices viz., **3.1-3.4** as well as unbound neat DPV and mononuclear pV containing amino acid, di- or tripeptide viz., **3.5** and **4.1-4.3** upon activity of rabbit intestine alkaline phophatase was investigated using p-NPP as substrate and employing established enzyme assay system. The dose dependent effects of each of the pV compounds in comparison to the free ligands are presented in *Fig. 4.3* and *Fig. 4 4*. To quantify the inhibitory potential of the molecules, we determined the half-maximal inhibitory concentration (IC_{50}) for each inhibitor, which gave rise to a 50 % suppression of the original enzyme activity (Table 4.2, 4.3). From the data obtained it is evident that each of the tested macromolecular as well as mononuclear peroxovanadates behaved as active inhibitor of ALP. The observed trend also shows that monomeric pV complexes exert greater inhibitory effect compared to the corresponding polymer immobilized analogues. On the basis of the observed data and on comparing the IC_{50} values of the polymeric compounds, in terms of their actual pV loading, with those of free pV compounds the inhibitors could be arranged in the following order of potency **pV1> pV2> pV4> pV3>DPV> PMAV> PSSV> PSS-*co*-MV> PAV.** It was observed that IC_{50} value obtained for **PMAV** was close to that of neat **DPV**. The effect of each of the polymeric ligands, without pV loading and amino acids and peptides viz., asparagine, glutamine, gly-gly or trigycine, upon ALP activity is practically negligible under the assay conditions used and H_2O_2 as such had no observable effect

The inhibitor efficiency of the macromolecular complexes with respect to the IC_{50} values are relatively less than that of DPV while mononuclear heteroligand diperoxovandate are stronger inhibitor than DPV. In case of macromolecular pV,

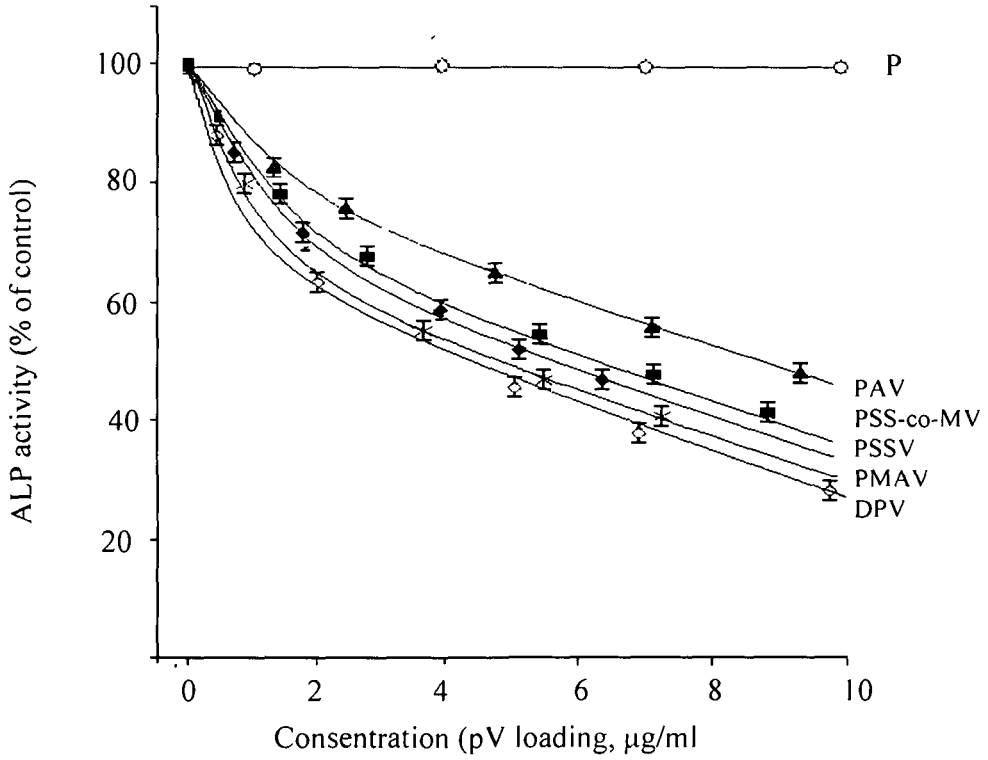

Fig. 4.3 Effect of compounds **PSSV, PSS-*co*-MV, PAV, PMAV, DPV** and free polymers (**P**) on activity of ALP from rabbit intestine. The ALP catalyzed rates of hydrolysis of p-NPP at pH 10.0 were determined at 30 ^{0}C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/ml), p-NPP (2 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) in the absence or presence of stated concentrations of the inhibitors. Effects of the additions are represented as the percent values (rounded to integers) of control (Δp-NPP = 3.13 μM/min). The data are presented as the means ± SE from three separate experiments.

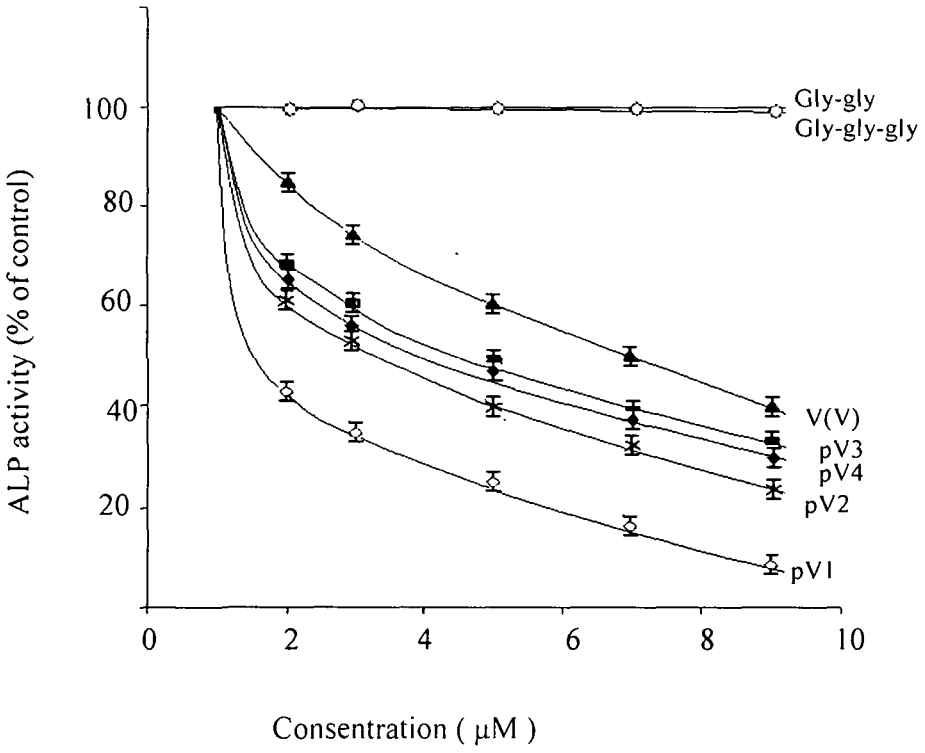

Fig. 4.4 Effect of compounds **pV1, pV2, pV3, pV4** and free ligand on activity of ALP from rabbit intestine. The ALP catalyzed rates of hydrolysis of p-NPP at pH 10.0 were determined at 30 ^{0}C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/ml), p-NPP (2 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) in the absence or presence of stated concentrations of the inhibitors. Effects of the additions are represented as the percent values (rounded to integers) of control (Δp-NPP = 3.13 μM/min). The data are presented as the means ± SE from three separate experiments.

compounds comparing the IC_{50} values in terms of actual pV loading in the polymeric compounds it was observed that value obtained for **PMAV** was close to that of free DPV.

An inhibitor species can interact with enzymes in various ways and enzyme kinetics investigation is a major tool in enabling us to distinguish between the inhibition mechanisms of enzyme catalyzed reactions. In order to examine the inhibitory mode of action of the complexes on ALP activity, we have determined kinetic parameters K_m and V_{max} in absence as well as in presence of peroxo vanadium compounds using Lineweaver-Burk double reciprocal plots. Presented in Table 4.2 are the kinetic data for inhibition of ALP catalysed hydrolysis of p-NPP by the macromolecular pV compounds and free DPV. Kinetic measurements at several different substrate concentrations in presence of each the inhibitors yielded straight lines with a point of intersection in the second quadrant (*Fig.4.5-4.8*). With an increase in concentration of each of the polymeric inhibitor complexes from 2-50 μg/ml, a decrease in velocity V_{max} was noted, whereas K_m remained constant. That the polymeric compounds are non-competitive inhibitors of ALP thus became self-evident from the data. From similar studies carried out using **DPV,** or any of the other four non-polymeric complexes, as inhibitor it was found that with increasing inhibitor concentration V_{max} decreased whereas K_m value increased (*Fig. 4.9-4.12*). From these changes in the V_{max} as well as K_m values it is apparent that **DPV** as well as each of the heteroligand peroxo vanadium compounds tested served as mixed-type of inhibitor of ALP combining competitive and non-competitive modes of inhibition.

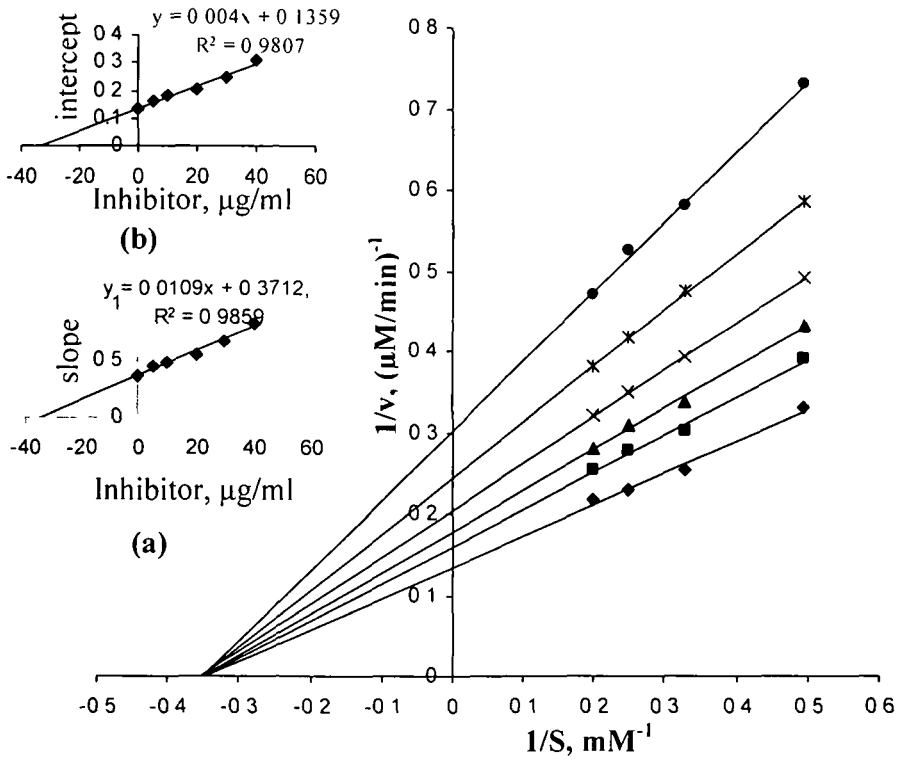

Fig. 4.5 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of (A) **PAV** . The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and *p*-NPP (2-5 mM). The reaction was started by adding ALP (3.3 µg/ml) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 µg/ml; ■ 5 µg/ml ▲ 10 µg/ml; × 20 µg/ml; * 30 µg/ml • 40 µg/ml inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset, Secondary plot of initial kinetic data of Lineweaver plot (a) The Slopes were plotted against inhibitor concentrations and K_i values were obtained from the x-intercepts of these replots. (b) The vertical intercepts were plotted against inhibitor concentration and K_{ii} values were obtained from the x-intercepts of these replots.

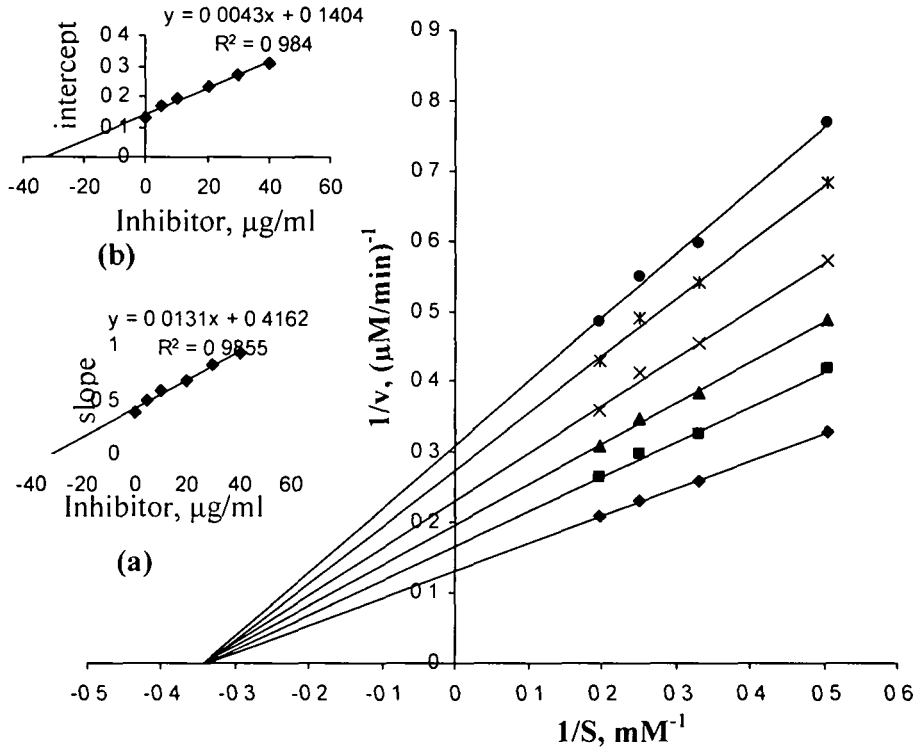

Fig. 4.6 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of (A) **PMAV** . The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and *p*-NPP (2-5 mM). The reaction was started by adding ALP (3.3 µg/ml) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 µg/ml; ■ 5 µg/ml ▲ 10 µg/ml; × 20 µg/ml; * 30 µg/ml • 40 µg/ml inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset, Secondary plot of initial kinetic data of Lineweaver plot (a) The Slopes were plotted against inhibitor concentrations and K_i values were obtained from the x-intercepts of these replots. (b) The vertical intercepts were plotted against inhibitor concentration and K_{ii} values were obtained from the x-intercepts of these replots.

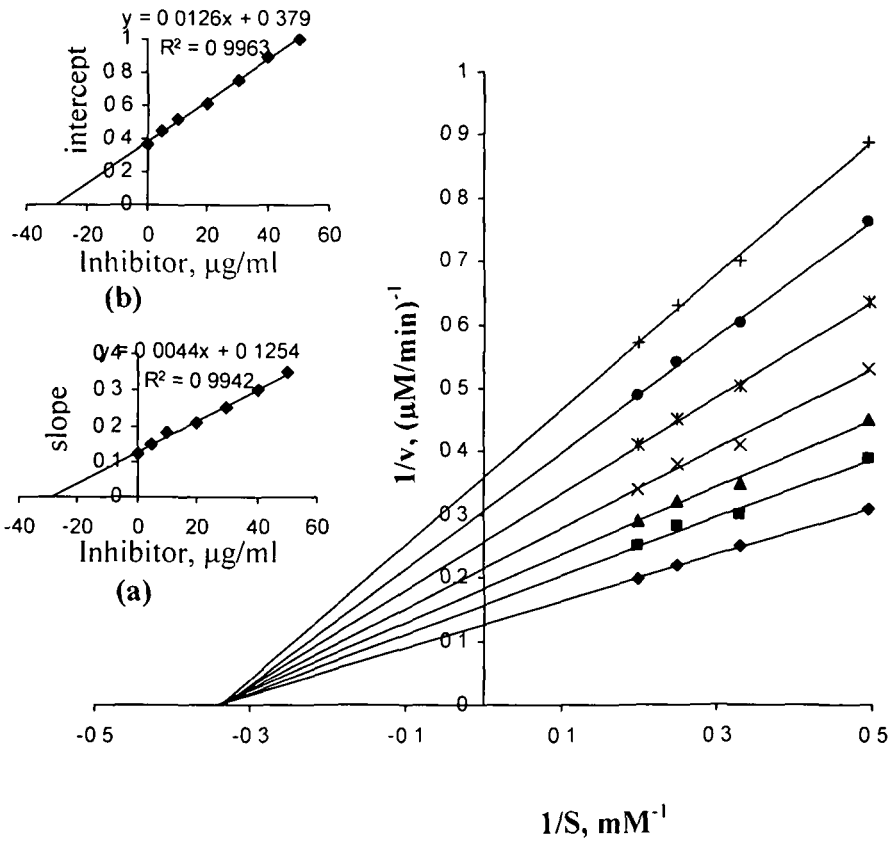

Fig. 4.7 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of (A) **PSSV** . The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and *p*-NPP (2-5 mM). The reaction was started by adding ALP (3.3 µg/ml) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 µg/ml; ■ 5 µg/ml ▲ 10 µg/ml; × 20 µg/ml; * 30 µg/ml • 40 µg/ml inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset, Secondary plot of initial kinetic data of Lineweaver plot (a) The Slopes were plotted against inhibitor concentrations and K_I values were obtained from the x-intercepts of these replots. (b) The vertical intercepts were plotted against inhibitor concentration and K_{II} values were obtained from the x-intercepts of these replots.

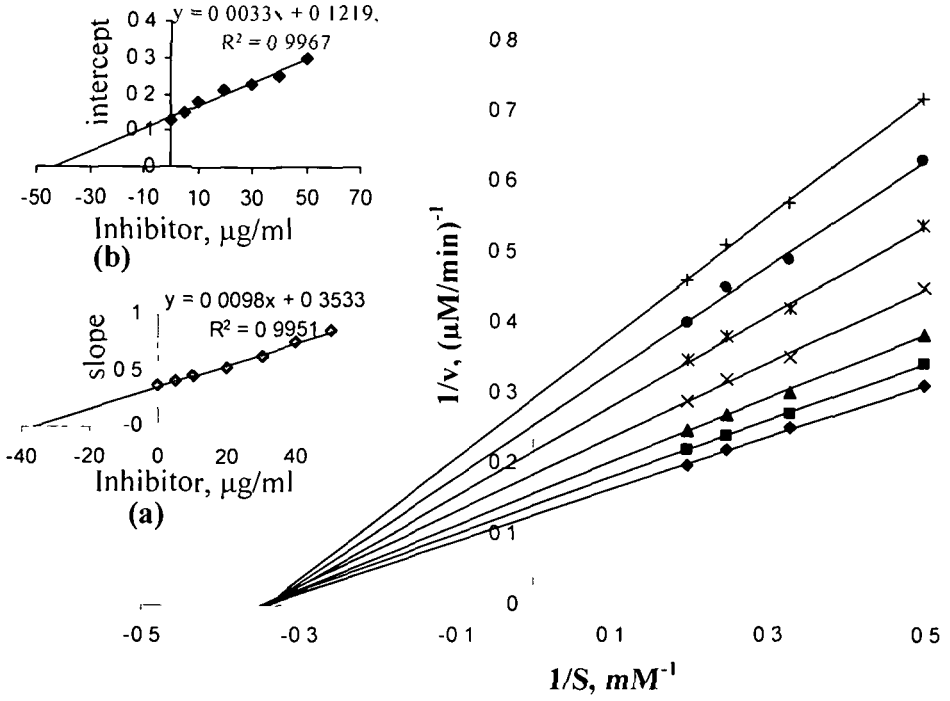

Fig. 4.8. Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of (A) **PSS-co-MV** . The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and *p*-NPP (2-5 mM). The reaction was started by adding ALP (3.3 µg/ml) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 µg/ml; ■ 5 µg/ml ▲ 10 µg/ml; × 20 µg/ml; * 30 µg/ml • 40 µg/ml inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset, Secondary plot of initial kinetic data of Lineweaver plot (a) The Slopes were plotted against inhibitor concentrations and K_I values were obtained from the x-intercepts of these replots. (b) The vertical intercepts were plotted against inhibitor concentration and K_{II} values were obtained from the x-intercepts of these replots.

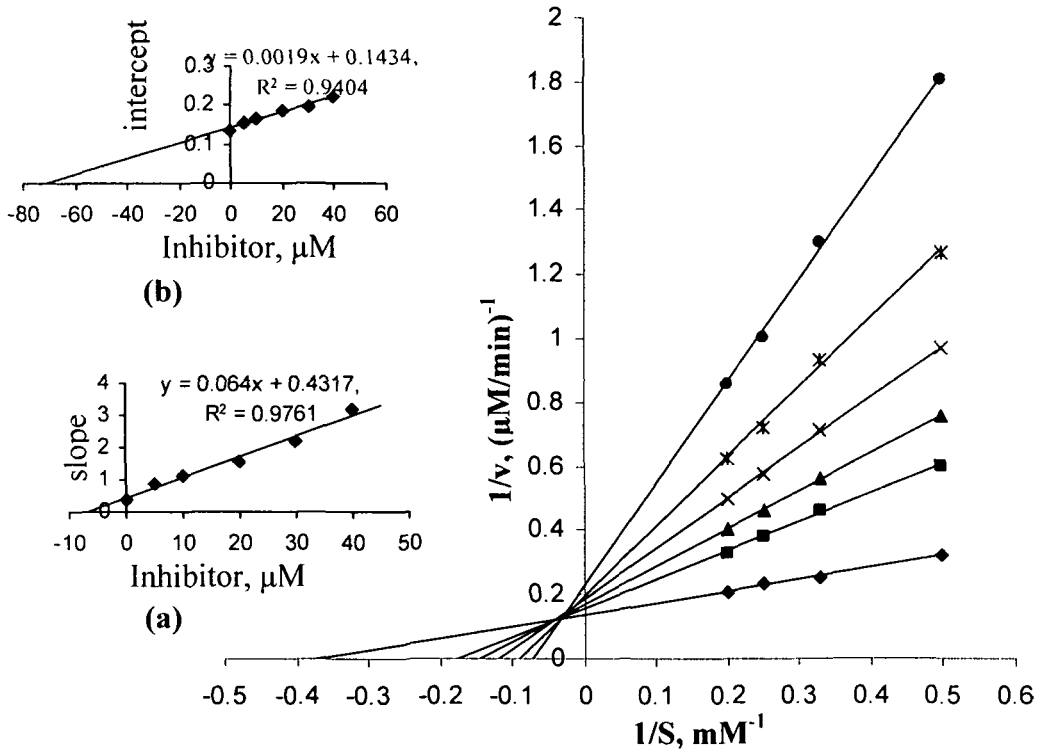

Fig. 4.9 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of **pV1** . The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and *p*-NPP (2-5 mM). The reaction was started by adding ALP (3.3 μg/ml) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 5 μM; ▲ 10 μM; × 20 μM; * 30 μM • 40 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset, Secondary plot of initial kinetic data of Lineweaver plot (a) The Slopes were plotted against inhibitor concentrations and K_i values were obtained from the x-intercepts of these replots. (b) The vertical intercepts were plotted against inhibitor concentration and K_{ii} values were obtained from the x-intercepts of these replots.

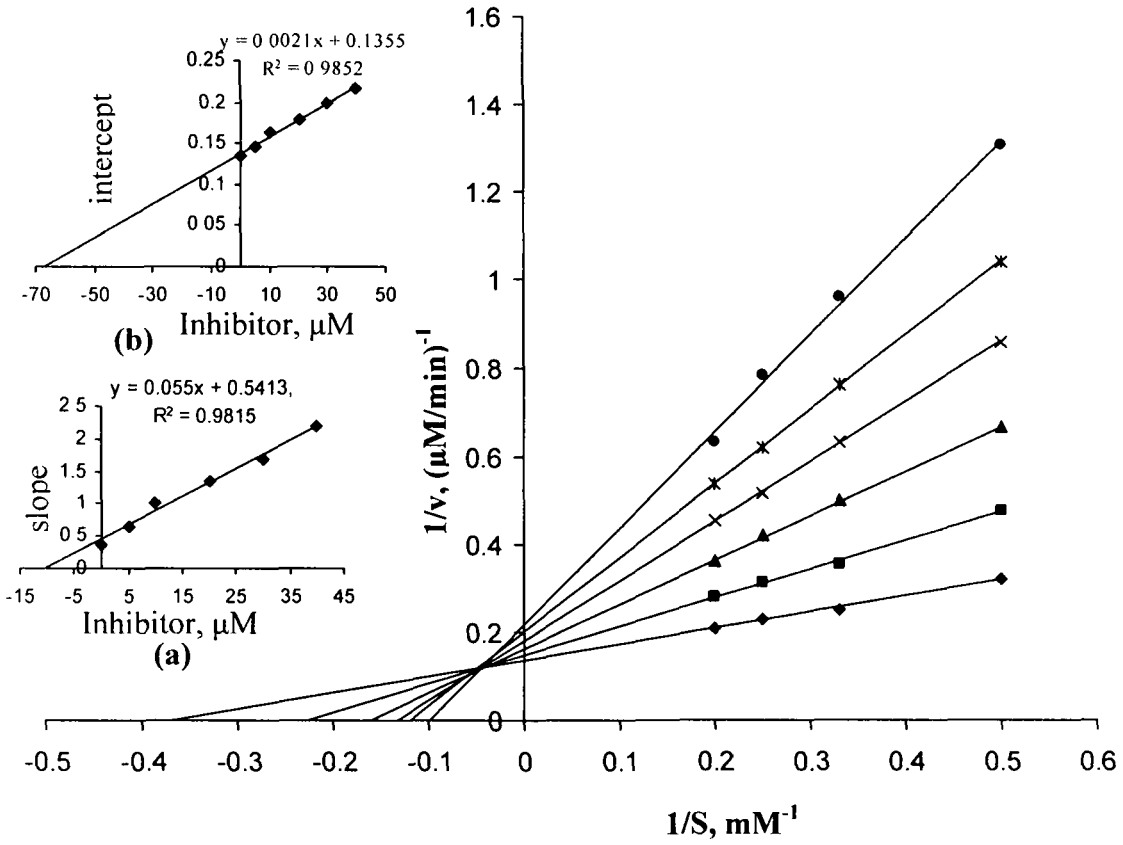

Fig. 4.10 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of **pV2** . The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and *p*-NPP (2-5 mM). The reaction was started by adding ALP (3.3 μg/ml) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 5 μM; ▲ 10 μM; × 20 μM; * 30 μM • 40 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset, Secondary plot of initial kinetic data of Lineweaver plot (a) The Slopes were plotted against inhibitor concentrations and K_i values were obtained from the x-intercepts of these replots. (b) The vertical intercepts were plotted against inhibitor concentration and K_{ii} values were obtained from the x-intercepts of these replots.

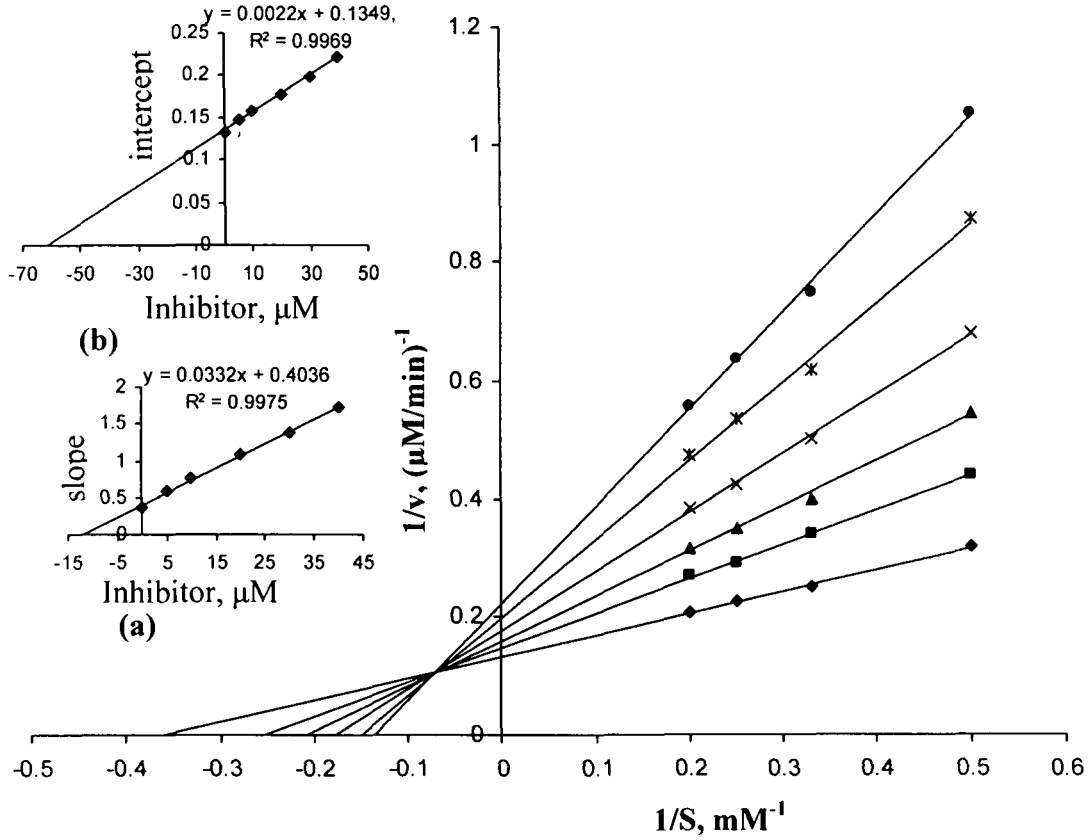

Fig. 4.11 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of **pV4** . The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and *p*-NPP (2-5 mM). The reaction was started by adding ALP (3.3 μg/ml) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 5 μM: ▲ 10 μM; × 20 μM; * 30 μM • 40 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset, Secondary plot of initial kinetic data of Lineweaver plot (a) The Slopes were plotted against inhibitor concentrations and K_i values were obtained from the x-intercepts of these replots. (b) The vertical intercepts were plotted against inhibitor concentration and K_{ii} values were obtained from the x-intercepts of these replots.

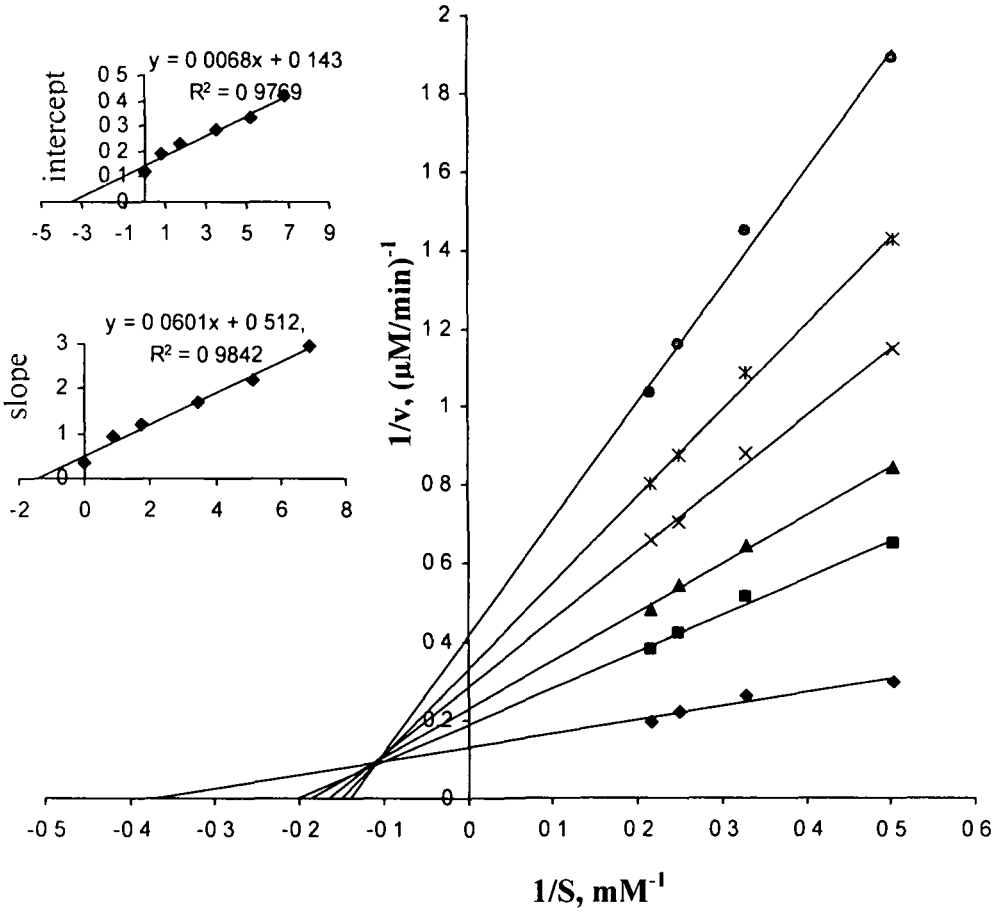

Fig. 4.12 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of ~ ÞpV , . The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and *p*-NPP (2-5 mM). The reaction was started by adding ALP (3.3 μg/ml) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 5 μM, ▲ 10 μM; × 20 μM; * 30 μM • 40 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset, Secondary plot of initial kinetic data of Lineweaver plot (a) The Slopes were plotted against inhibitor concentrations and K_i values were obtained from the x-intercepts of these replots. (b) The vertical intercepts were plotted against inhibitor concentration and K_{ii} values were obtained from the x-intercepts of these replots.

Table 4.2

Half-maximal inhibitory concentration (IC_{50}), and inhibitor constants values of **3.1-3.4** and other inhibitors against ALP

Inhibitor	IC_{50} (μg/ml)	K_I(μg/ml)	K_{II}(μg/ml)	K_{II}/K_I	Type of inhibition
	(containing μg equivalent of pV)				
1.**PSSV**	45.26 (5.86)	30.00 (3.88)	29.50 (3.81)	0.98	noncometitive
2.**PSS-*co*-MV**	33.68 (6.16)	38.45 (6.92)	38.28 (6.89)	0.99	noncompetitive
3.**PMAV**	26.00 (4.74)	31.00 (5.65)	30.50 (5.56)	0.98	noncompetitive
4.**PAV**	35.00 (8.33)	34.50 (8.21)	34.00 (8.09)	0.99	noncompetitve
5.**DPV**	4.33	1.57	3.65	2.4	Mixed inhibition
6.Free polymer	-	-	-	-	-

Note: The ALP catalyzed rates of hydrolysis of p-NPP at pH 10.0 were determined at 30 ^{0}C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/ml), p-NPP (2-5 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) in the presence of stated concentrations of the inhibitors (*Fig. 4.5-4.8*).The V_{max} and K_m in absence of inhibitor were found to be 7.9 μM/min and 2.85 mM. In presence of inhibitors V_{max} decreases and K_m = 2.85 remained constant.

Table 4.3

Half maximal inhibitory concentration $IC_{50}(\mu M)$ and inhibitor constant values for **3.5, 4.1-4.3** of ALP activities

	$IC_{50}(\mu M)$	$K_I(\mu M)$	$K_{II}(\mu M)$	K_{II}/K_I	Type of inhibition
1. PV1	2.5	6.0	71.0	11.83	Mixed inhibition
2. pV2	11.8	10.0	68.0	6.80	Mixed inhiition
3. pV3	16.5	12.0	61.0	5.08	Mixed inhibition
4. pV4	15.0	13.0	52.0	4.00	Mixed inhibition

Note: The ALP catalyzed rates of hydrolysis of p-NPP at pH 10.0 were determined at 30 ^{0}C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/ml), p-NPP (2-5 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) in the presence of stated concentrations of the inhibitors (*Fig. 4.9-4.12*).The V_{max} and K_m in absence of inhibitor were found to be 7.9 μM/min and 2.85 mM. In presence of inhibitors V_{max} decreases and $K_m$increases.

The extent of changes in the K_m or V_{max} values compared to that observed for the control brought about by the presence of an inhibitor, depends on the concentration of inhibitor used and also on the affinity of the enzyme for the inhibitor, which can be measured by inhibitor constants. The inhibitor constant K_I for competitive part of inhibition was determined from the secondary plot of slope of the primary plot (1/V versus 1/[S]) against the inhibitor concentration with intercept on the inhibitor axis being $-K_I$. The value of K_{II}, inhibitor constant for non-competitive inhibition, was obtained from a linear secondary plot of $1/V_{max}$ against the inhibitor concentration of each inhibitor, the intercept on the inhibitor axis being equivalent to $-K_{II}$. For each of the macromolecular complexes value of K_I is found to be equal to K_{II}, which is typical of a non-competitive inhibitor. For mononuclear diperoxovanadate, $K_{II} > K_I$ as is the case with a mixed type of inhibitor with major mode of inhibition being of competitive type.

Data obtained from similar experiments conducted with vanadate used as inhibitors showed that while V_{max} remained constatnt, K_m value increased with increasing inhibitor concentration. These observations are typical of a competitive inhibitor. The K_I value determined for vanadate is 15 μM.

4.4 DISCUSSION

Action of catalase on the newly synthesized compounds is a slow process in contrast to that of its natural substrate, H_2O_2. Under the effect of catalase the rate of degradation of H_2O_2 with the release of oxygen was reported to be 430 μM /min from a solution of 0.1 mM concentration[19]. It is thus evident that the synthesized polymer

anchored pV complexes[17] are at least 80-100 times weaker as substrates to catalase compared to H_2O_2. On the other hand the monomeric complex **3.5** like the other previously reported diperoxovanadate compounds is about 30 fold weaker substrate to the enzyme in comparison to H_2O_2. Thus it is evident that ability of the co-ordinated peroxo group to resist the action of catalase increases on co-ordination to V(V) and the extent of this resistance is sensitive to the other co-ligands present in the complex sphere. The relatively greater resistance of the polymeric compounds to the powerful enzyme catalase appears to be consequence of additional stability imparted to the compounds by the polymeric support.

One of the most noteworthy features emerging out of our data derived from kinetic studies on inhibition of ALP activity is that the mode of inhibition induced by macromolecular pV compounds is distinctly different from that of simple DPV as well as mononuclear heteroligand peroxovanadates. Each of the macromolecular peroxovanadate compounds is a classical non-competitive inhibitor of ALP whereas the non-polymeric pV tested exert mixed-type of inhibition on ALP with the K_i and K_{ii} values in the micromolar range. That the major mode of inhibition induced by these complexes is of competitive type is evident from the K_{ii}/ K_i ratios for the inhibitor species. The observation that vanadate is a competitive inhibitor of the ALP is in agreement with the earlier reports.

Previous studies indicated that oxyanions such as vanadate[9, 10, 13], molybdate and tungstate[20-22] are in general competitive inhibitors of the phosphatases. Such inhibition is attributed to the formation of pentaco-ordinated or hexaco-ordinated structures, which are often described as phosphate analogues[9-13,20-22]. It has been observed earlier that inhibitor potency of vanadium complexes depends on several factors such as oxidation state of the metal, co-ordination geometry, stability of the

compounds under physiological conditions, and the nature of the phosphoproteins[9,11,13]. Information available from the limited reports on ALP inhibitory activity on heteroligand pV compounds shows that majority of the compounds tested were competitive inhibitors[8] of the enzyme although, there are examples of diperoxovanadate compounds showing mixed-inhibition of Green-crab ALP, with K_I and K_{II} values in milimolar range[23]. From their investigation on inhibition of chicken intestinal ALP by six- and seven co-ordinated monoperoxo and diperoxovanadate compounds, Crans *et al.* observed that a co-relation exists between inhibitor potency and co-ordination geometry of the complexes which served as potent competitive inhibitors[9] of ALP Five coordinated compounds are documented to be more potent inhibitors than the 6 or 7 co-ordinated ones.

A significant observation of the present investigation is that although individually each of the tested species inhibited ALP activity to varying degrees, inhibitor potency of the intact pV compounds tested despite having hexa or hepta-coordinated metal center in each of them, is more than additive of the combined effect that may be expected of equivalent concentrations of the corresponding metalloxide or metal peroxide formed in solution.

It is also noteworthy that although the effect of the individual ligand on the ALP activity is practically negligible under the assay conditions used, our results show that there is a marked influence of the co-ligand environment on the inhibitory potency of the intact metal complexes as well as mode of their inhibition of the enzyme. Interestingly, IC_{50} value of **PMAV [3.2]** is almost half of that of **PAV [3.1]** in spite of these polymers having the similar carboxylate functional group bound to the vanadium centre. Such variation in inhibitor potency may be attributed to the difference in mode of co-ordination of pV in these compounds. Pertinent here is to

mention that the two compounds also showed remarkable difference in their redox activity in oxidative bromination, as will be seen in Chapter 6 of the present thesis. In **PMAV [3.2]** and **PSSV [3.2]** the diperoxovanadate moieties are bound exclusively in a monomeric fashion whereas in **PAV [3.1]** the pV occur as dinuclear tetraperoxo species and in the compound **PSS-*co*-MV [3.4],** presence of both dimeric as well as monomeric pV units are observed to be present.

It is known that a competitive inhibitor typically has close structural similarities to the normal substrate for the enzyme. A non-competitive inhibitor binds reversibly at a site other than the active site and causes a change in the overall three-dimensional shape of the enzyme that leads to a decrease in catalytic activity. In the present study, factors such as structural analogy with the transition state or phosphate mimicry are unlikely to play a role in the non-competitive inhibition exhibited by the macromolecular complexes. Also since each of the compounds is 7-coordinated, it may be anticipated that the inhibitory effect of the complexes would be due to some additional factors other than structural analogy with the transition state.

Due to the complexity of the reaction and species involved it is difficult to draw any conclusion regarding exact mechanism of inhibition of ALP by the compounds tested. Nevertheless, considering our observation in conjunction with the reports documenting the importance of redox properties of peroxo vanadium compounds in inhibition of protein phosphatases[9, 13], it is reasonable to expect that the redox interaction of the complex with the enzyme should be one of the possible factors responsible for making the compounds under investigation effective non-competitive inhibitors of the phosphoproteins. On the other hand, both of these factors viz., transition state analogy as well as oxidant activity of the peroxovanadate species are likely to contribute to the mixed type of inhibition, combining competitive and

non-competitive pathways, exhibited by the hexa coordinated neat DPV complex and the monomeric pV compounds. However, it is also likely that some other mechanism of inhibition is operative in the present case which is yet to be unraveled.

In conclusion, the present experiments establish that the peroxovandate anchored to soluble polymers as well as free diperoxovandadate and its derivatives with amino acid or small peptide as ancillary ligand, induces strong inhibitory effect on rabbit intestine alkaline phosphatase. Significantly, macromolecular compounds are non-competitive inhibitor of the enzyme whereas non-polymeric diperoxovandates are of mixed type inhibitor of the ALP. It is also noteworthy that the title compounds are relatively resistant to degradation by the powerful enzyme catalase. This may be relevant in the cellular milieu where H_2O_2 has little chance to survive abundant catalase. Our results also demonstrate that there is a marked influence of the co-ligand and mode of attachment of the pV moiety to the polymer chain on the bioactivity of the pV compounds.

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CHAPTER 5

5.1 INTRODUCTION

Among the various types of metal complexes studied for their pharmacological potential, vanadium and its peroxo compounds are gaining a special status in medicinal inorganic chemistry owing mainly to their antineoplastic activity[1-5] and well-established role as insulin-mimetic agents[5-17,18]. Research directed toward specific and selective functionality of vanadium complexes of numerous types are leading to potent drugs for the treatment of diseases like diabetes and cancer[1-17]. Also, as more knowledge about the effects of vanadium in biological systems are being unravelled, other applications in medicine may be found for the metal, including the development of vanadium based antibacterial drugs for the treatment of burn victims[18,19]. This is an area in which silver containing compounds have achieved great success as antibacterial agents[20]. Some vanadium compounds including decavanadate[21-23] and haloperoxidase[24], isolated from filamentous fungus Curvularia verruculosa were reported to exhibit antibacterial activity. Moreover, the antibacterial properties of hydrogen peroxide against Gram - positive (G +ve) and Gram - negative (G –ve) bacteria have been well established[25]. It is therefore surprising that no report seems to exist on antibacterial effect of peroxovanadates despite the existing awareness regarding therapeutic potential of such compounds.

Results described in this chapter have been published [illegible]
React. Funct. Polym., 2008, 68, 876.

The above observations and our specific interest on biological activity of peroxo compounds of vanadium(V), provided us with much of the impetus to explore the effect of a range of pV compounds including neat DPV, monomeric heteroligand diperoxovanadates and newly synthesized polymeric complexes, on growth of gram positive and gram negative bacteria. Also, in view of the increasing current interest in the development of pharmaceutical formulation consisting of acrylic acid [26,27,28] and sulphonic acid based polymers[29], the soluble polymeric complexes viz., (**PAV**) [**3.1**] and (**PMAV**) [**3.2**], (**PSSV**) [**3.3**] and (**PSS-co-MV**) [**3.4**] in particular, appeared to be promising candidates for the present investigation. It has been found recently that polystyrene sulphonate is effective in treating antibiotic- associated diarrhea and it finds application as a noncytotoxic antimicrobial contraceptive agent[29]. Binding of active drug molecules including low molecular weight metal complexes to soluble macromolecular carriers are receiving importance since such systems can be expected to overcome the limitation such as toxic side effects by improving the body distribution of drugs and prolonging their activity [30-33].

Reported in this chapter are the results of antibacterial screening of macromolecular peroxovanadium $[V_2O_2(O_2)_4(carboxylate)]$-PA [PA = poly(acrylate)] (**PAV**) [**3.1**] and $[VO(O_2)_2(carboxylate)]$-PMA [PMA = poly(methacrylate)] (**PMAV**), [**3.2**], $[VO(O_2)_2(sulfonate)]$-PSS [PSS = poly(sodium 4-styrene sulfonate)] (**PSSV**) [**3.3**] and $[V_2O_2(O_2)_4(carboxylate)VO(O_2)_2(sulfonate)]$-P(SS-*co*-M) [P(SS-*co*-M)= poly(sodium styrene sulfonate-*co*-maleate)] (**PSS-co-MV**) [**3.4**] and mononuclear complexes, $Na[VO(O_2)_2(gln)].H_2O$ [**4.1**] (gln = glutamine), $Na[VO(O_2)_2(asn)].H_2O$ [**4.2**] (asn = asparagine) against gram- negative *E. coli* and

gram - positive *S. aureus*. The bactericidal effect of these compounds have also been compared with the unbound neat diperoxovanadate complex species (DPV).

5.2 EXPERIMENTAL SECTION

5.2.1 Assessment of antibacterial activity

For the antibacterial activity *E. coli* MG1655 was grown and maintained in Luria Agar. Culture was transferred 6-8 h prior to use to Luria Broth and incubated at 37 ^{0}C . *S. aureus* MTCC96 was grown and maintained in Nutrient Agar. Culture was transferred 6-8 prior to use to Nutrient Broth and incubated at 37 ^{0}C.

Antibacterial activity against *S. aureus* (gram -positive) and *E. coli* (gram - negative) were determined by the optical method and method of plate counting. Typically 1 ml of bacterial suspension containing 2-5 × 10^6 cells/ ml were incubated with either medium alone (positive control) or predetermined concentration of test compound in a final volume of 1 ml at 37 ^{0}C for 45 min. Thereafter the cells were diluted and absorbance at 620 was measured and then plated onto agar plates and CFU counted after overnight incubation at 37 ^{0}C. The decrease in absorbance in presence of the complexes indicate the inhibition of growth of bacteria. The antibacterial effect estimated by calculating % inhibition using the formula

$$\%\text{inhibition} = \left(\frac{C-T}{C}\right)\times 100$$

where C = mean of CFU in control and T = mean of CFU in presence of given concentration of test compounds. The number of CFU was calculated by multiplying the numbers of colonies with the dilution factor.

The MIC values were determined as the minimum concentration of the inhibitor compound at which complete inhibition of the bacterial growth was observed in comparison to the control, disregarding single colony or a faint haze caused by the inoculums

5.2.2 Sphaeroplasting of bacterial cells

Briefly *E. coli* and *S. aureus* were cultured in Luria broth and Nutrient Broth respectively for 6-8 hrs and cells were collected by centrifugation. The cells were then washed with phosphate Buffer and treated with lysozome. Loss of cell wall was monitored by microscopic analysis. The sphaeroplasted cells were washed in Phosphate Buffer and resuspended to concentration 1-2× 10^6 cells/ml and used for assay as stated in the section [5.21]

5.2.3 RESULTS AND INTERPRETATION

The effect of macromolecular pV compounds **3.1-3.4**, mononuclear pV compounds **4.2** and **4.3** and free DPV on growth of bacteria viz., gram-negative *E. coli* and gram-positive *S. aureus* was examined by the method of viable counting as well as by monitoring the change in optical density of cultured medium at 620 nm (A_{620}). In presence of each of the compounds the number of CFU was found to be reduced considerably with respect to the control from which percent inhibition was calculated and compared with the effect induced separately by the free DPV, amino acids ligands as well as polymeric supports.

A significant decrease in number of colony forming units (CFU) of *E. coli* ($p<0.05$) as well as of *S. aureus* ($p<0.005$) was noted at concentrations ≥ 100 μg/ml of **PAV** (*Fig.5.1 and 5.2*). The minimal inhibitory concentration (MIC) of acrylate based polymer **PAV** calculated from the data for *E. coli* and of *S. aureus* was 375 μg/ml and 500 μg/ml. Significantly, **PMAV** treatment resulted in 66.8% inhibition of *S. aureus* at concentration of 20 μg/ml but equivalent inhibition of *E. coli* was seen at concentrations greater than 125 μg/ml. Although, the two bacteria responded differently to lower concentrations of **PMAV**, the MIC for both bacteria was noted to be 375 μg/ml. Further, this antibacterial activity against *E. coli* was specific. A gradual decrease in absorbance at 620 nm and CFU of *E. coli* ($p<0.005$) was observed in presence of the compound **PSSV(3.3)** or **PSS-co-MV(3.4)** in the concentration range of 20-650 μg/ml (*Fig. 5.3 and 5.4*).

The minimal inhibitory concentration (MIC) of both the polymeric compounds **PSSV** and **PSS-co-MV** calculated from the data was 650 μg/ml. The inhibitory effect of these compounds both in terms of rate of inhibition as well as MIC values on the growth of *S. aureus* ($p<0.03$) was observed to be significantly greater in comparison to *E. Coli* as evident from the data in Table 5.1. Difference was however noted in the inhibition profile and the MIC values between the two polymeric complexes with respect to their effect on *S. aureus*. It is notable that free DPV showed antibacterial activity for both *E. coli* and *S. aureus*. Under our experimental conditions used the effect of free polymers on bacterial growth was practically negligible.

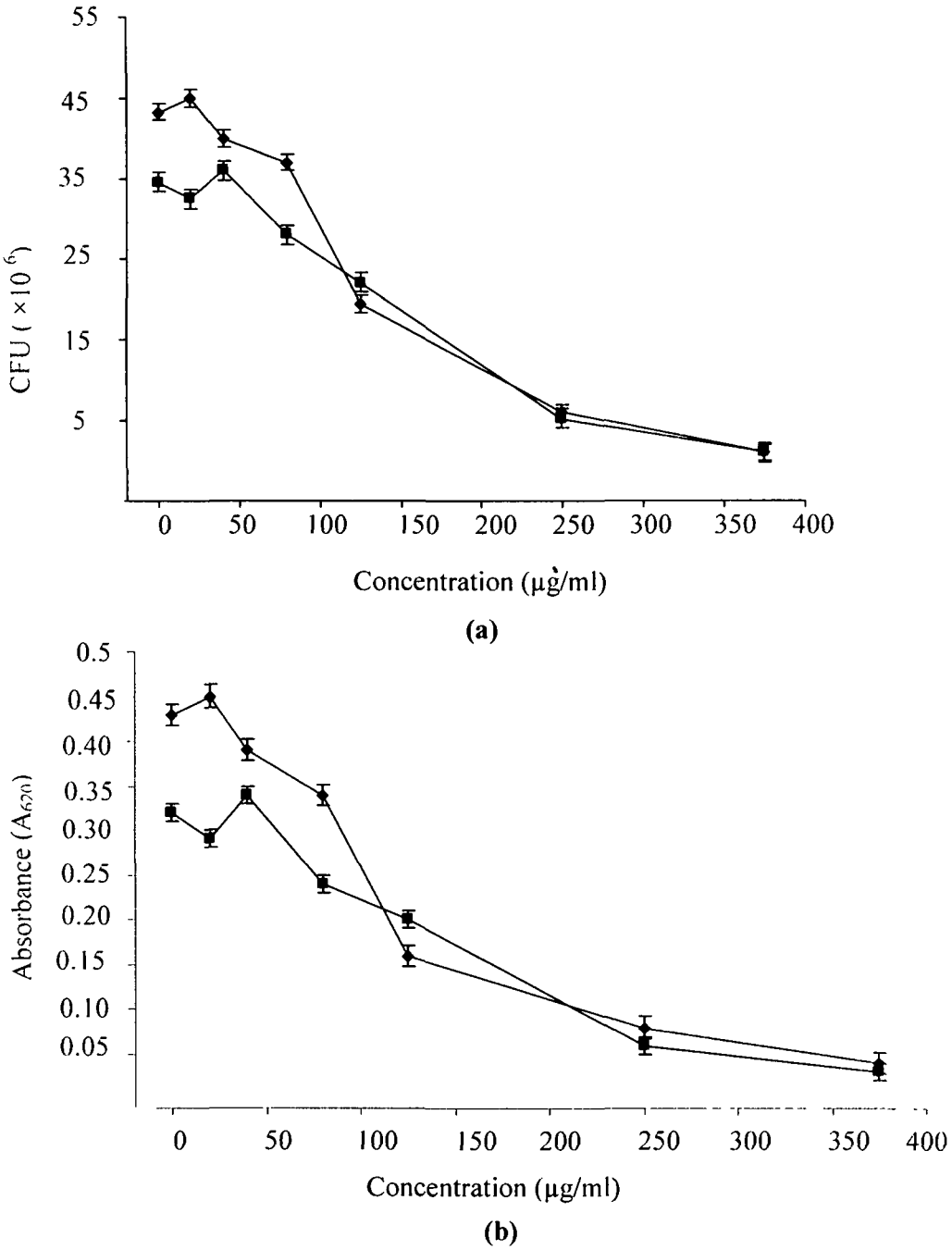

Fig. 5.1 Antibacterial activity of the compounds **PAV(♦)** and **PMAV(■)** against *E.coli* .(a) CFU and (b) Absorbance at 620 nm(A_{620}) against the inhibitor concentration. The data were the means ± SE of three parallel experiments that were done in triplicate.

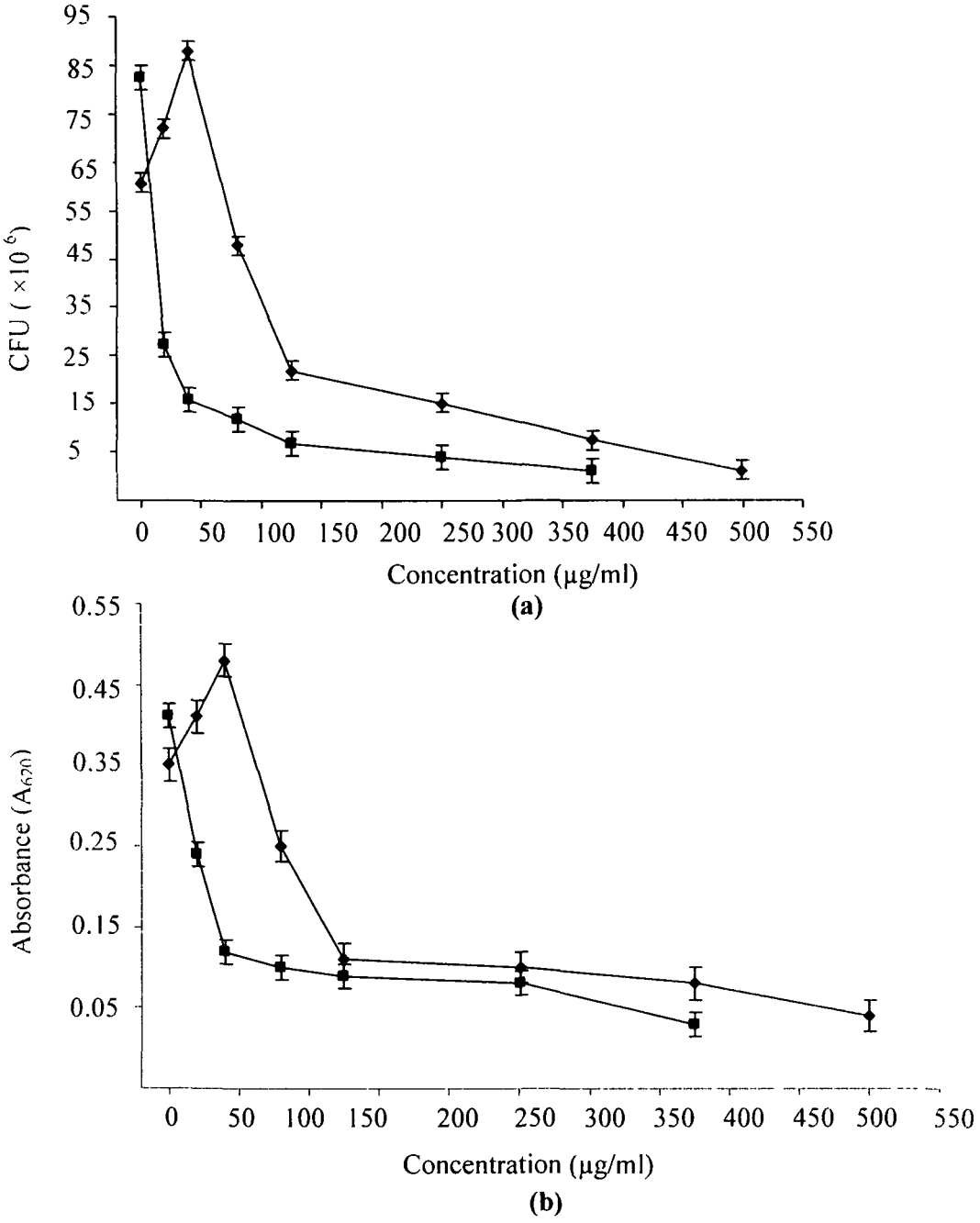

Fig. 5.2 Antibacterial activity of the compounds **PAV(♦)** and **PMAV(■)**against *S aureus* .(a) CFU and (b) Absorbance at 620 nm(A_{620}) against the inhibitor concentration. The data were the means ± SE of three parallel experiments that were done in triplicate.

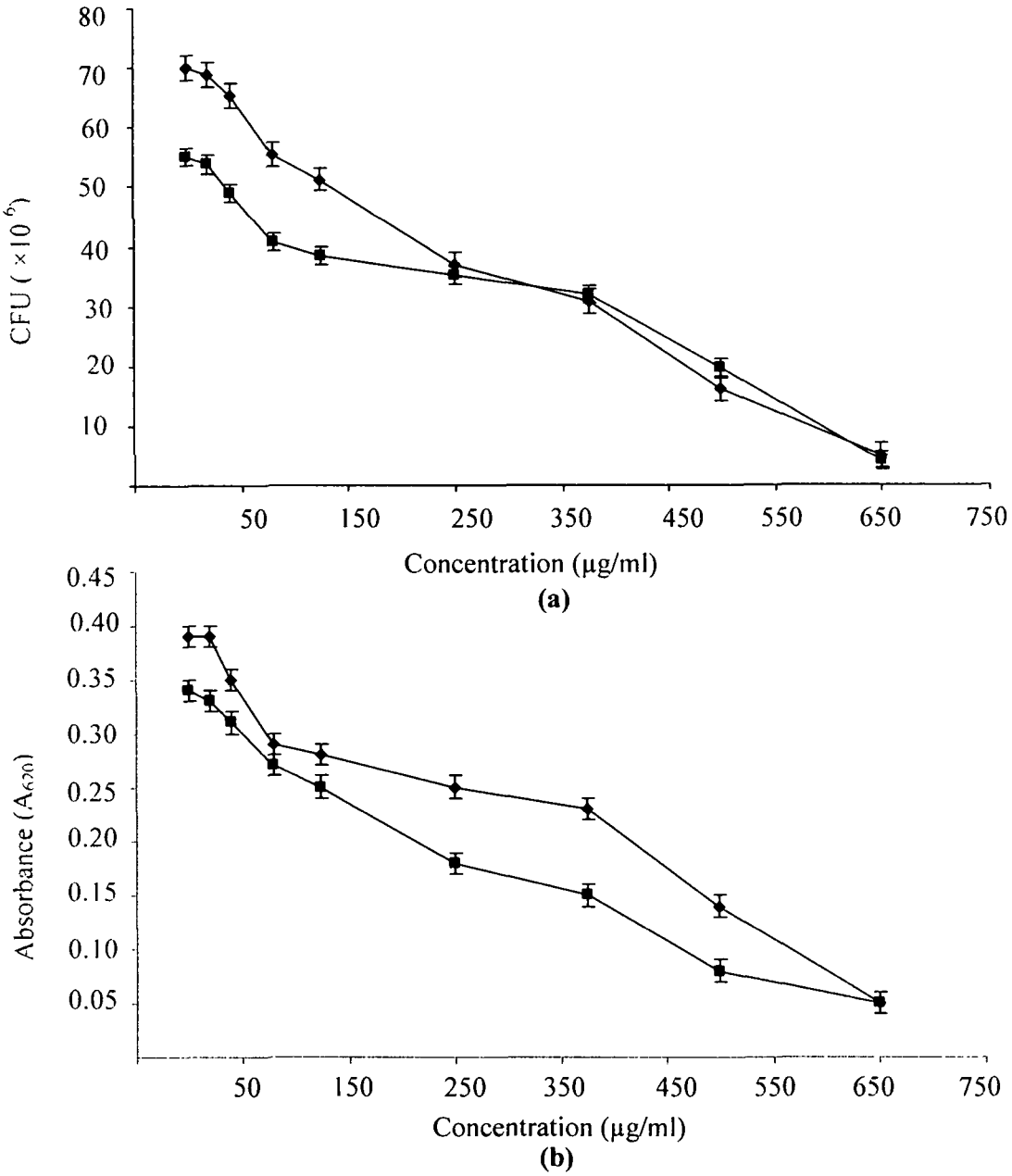

Fig.5.3 Antibacterial activity of the compounds **PSS-co-MV(■)** and **PSSV(♦)** against *E.coli* .(a) CFU and (b) Absorbance at 620 nm(A_{620}) against the inhibitor concentration. The data were the means ± SE of three parallel experiments that were done in triplicate

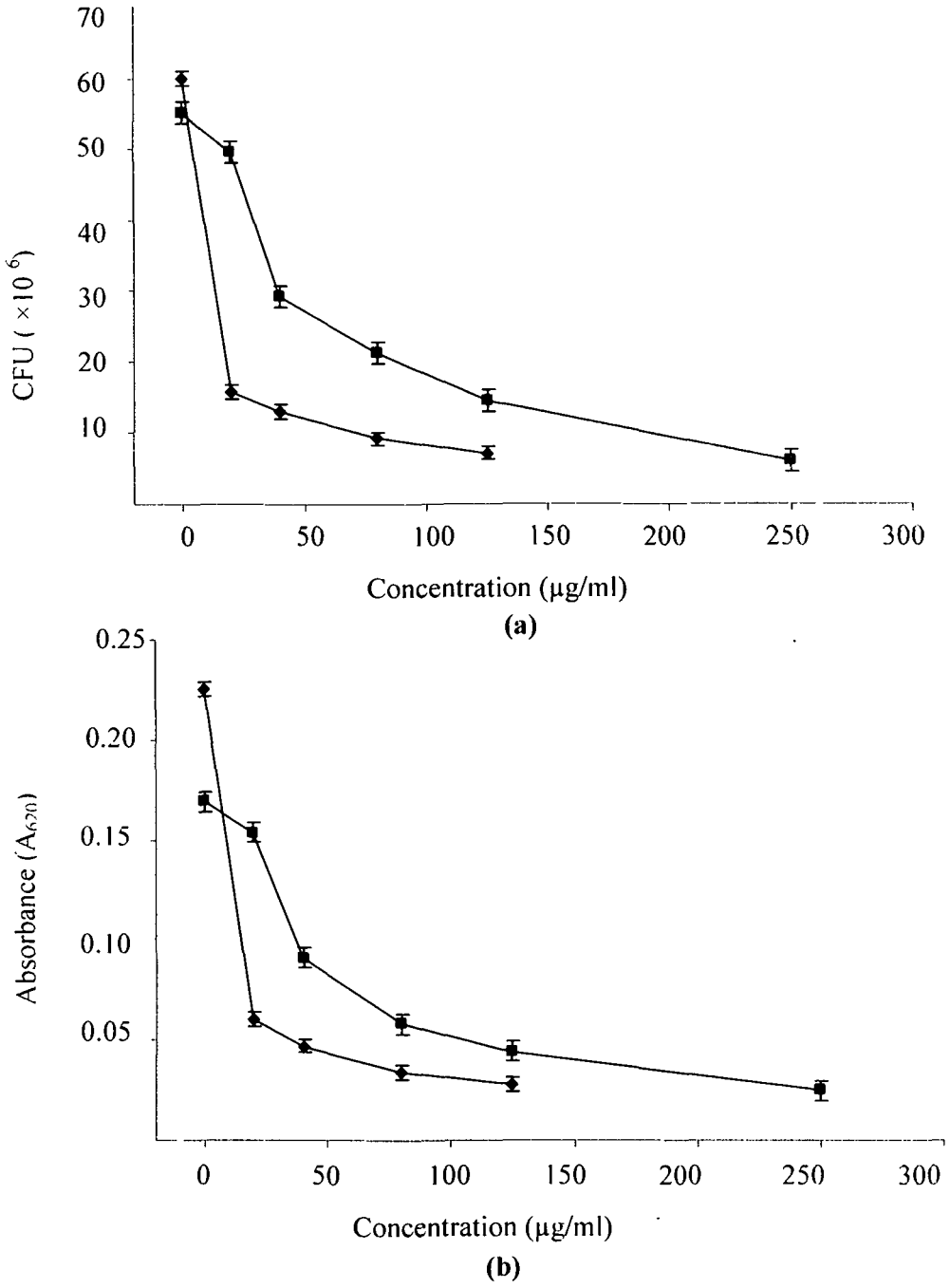

Fig. 5.4 Antibacterial activity of the compounds **PSS-co-MV(■)** and **PSSV (♦)** against *S aureus* .(a) CFU and (b) Absorbance at 620 nm(A_{620}) against the inhibitor concentration. The data were the means ± SE of three parallel experiments that were done in triplicate

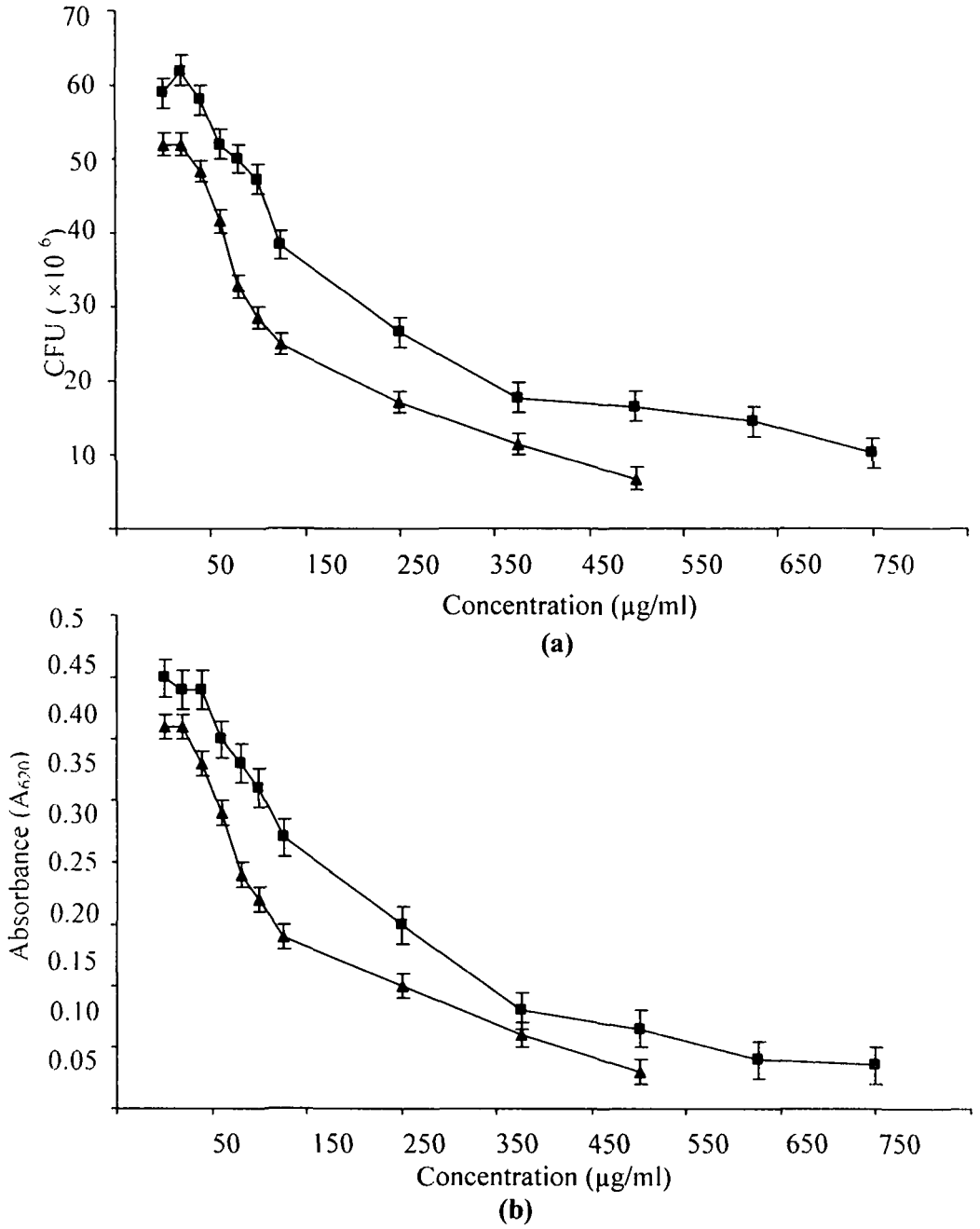

Fig. 5.5 Antibacterial activity of the compounds **pV3(▲)** and **pV4(■)** against *E.coli* .(a) CFU and (b) Absorbance at 620 nm(A_{620}) against the inhibitor concentration. The data were the means ± SE of three parallel experiments that were done in triplicate.

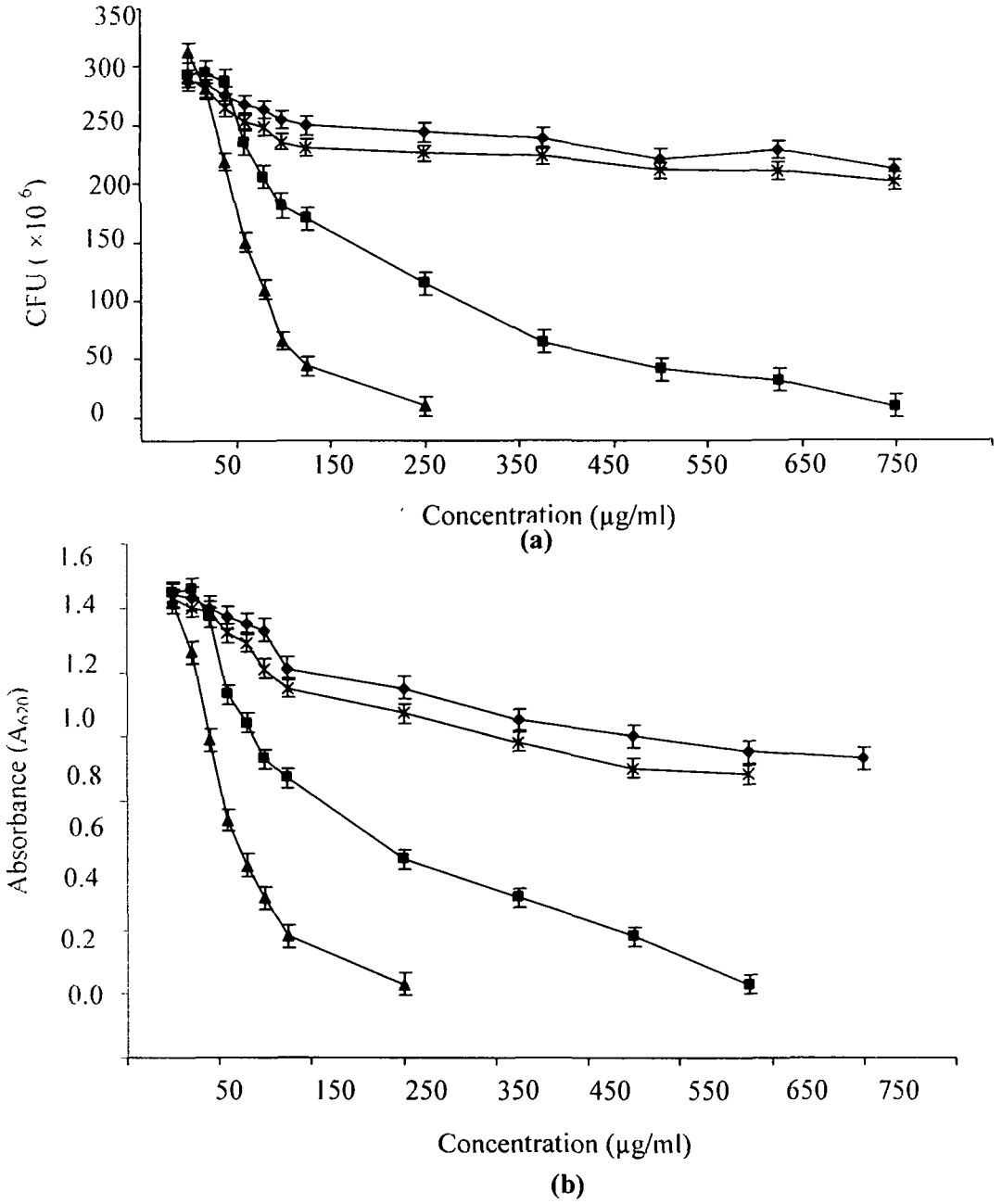

Fig. 5.6 Antibacterial activity of the compounds **pV3 (▲)** and **pV4 (■)**, vanadate (●), glutamine(×) against *S. aureus* .(a) CFU and (b) Absorbance at 620 nm(A_{620}) against the inhibitor concentration. The data were the means ± SE of three parallel experiments that were done in triplicate

Table 5.1 Antibacterial activity of the complexes against *E coli* and *S. aureus*

Compound	MIC (μg/ml) (containing μg equivalent of pV)[a]	
	E. coli	*S. aureus*
1. **PAV [3.1]**	375 (89.2)	500 (119.0)
2. **PMAV [3.2]**	375 (68.5)	375 (68.5)
3 . **PSSV [3.3]**	650 (84.5)	125 (16.2)
4. **PSS-*co*-MV[3.4]**	650.0 (117.0)	250 (45.0)
5.**pV3 [4.2]**	500 (161)[b]	250 (80.5)[b]
6.**pV4 [4.3]**	>750 -	750 (238)[b]
3. **DPV**	129.0	129.0

[a] calculated on the basis of pV loading
[b] concentration in μM

The gradual decrease in the number of CFU that has been observed in presence of mononuclear heteroligand pV compounds **4.2 (pV3)** and **4.3 (pV4)** are shown in *Fig. 5.5-5.6*. Both the compounds tested were noted to be more effective in comparison to vanadate or the free amino acid ligands viz., Gln or Asn, in the inhibtition of bacterial growth at all the concentrations tested. The minimal inhibitory concentration (MIC) of pV3 were found to be 500μM and 250μM for *E. coli* and of *S. aureus*, respectively. **pV3** treatment resulted ~50% inhibition of *S. aureus* at concentration of 60 μM whereas equivalent inhibition of *E. coli* was seen at concentrations greater than 125 μM. The MIC value for pV4 for *S. aureus* was

observed to be 750 μM however no 100 % inhibition of bacterial growth was observed in case of *E .coli* even at higher ≥750 μM. From the data obtained it is apparent that between the two mononuclear heteroligand peroxovanadate, **pV3** was significantly more inhibitory for both *S. aureus* and *E. coli* compared to **pV4**. However, these compounds are relatively less effective in comparison to polymer bound pV.

With an aim to understand the role of cell wall in the antibacterial activity of the peroxovanadate compounds the two organism were sphaeroplasted which were then treated with **pV3(4.1)**, taking as the representative compound. Although a significant difference between percent inhibition of *E. coli* and *S. aureus* was observed in presence of 250 μM of **pV3** treated whole cell of the organisms, this difference apparently disappeared after removal of cell wall from each of the organism (Fig. 5.7). The percentage inhibition obtained with sphaeroplast was significantly lower than that with whole cells. It was felt that this difference could be related to extracellular enzymatic degradation of the compound.

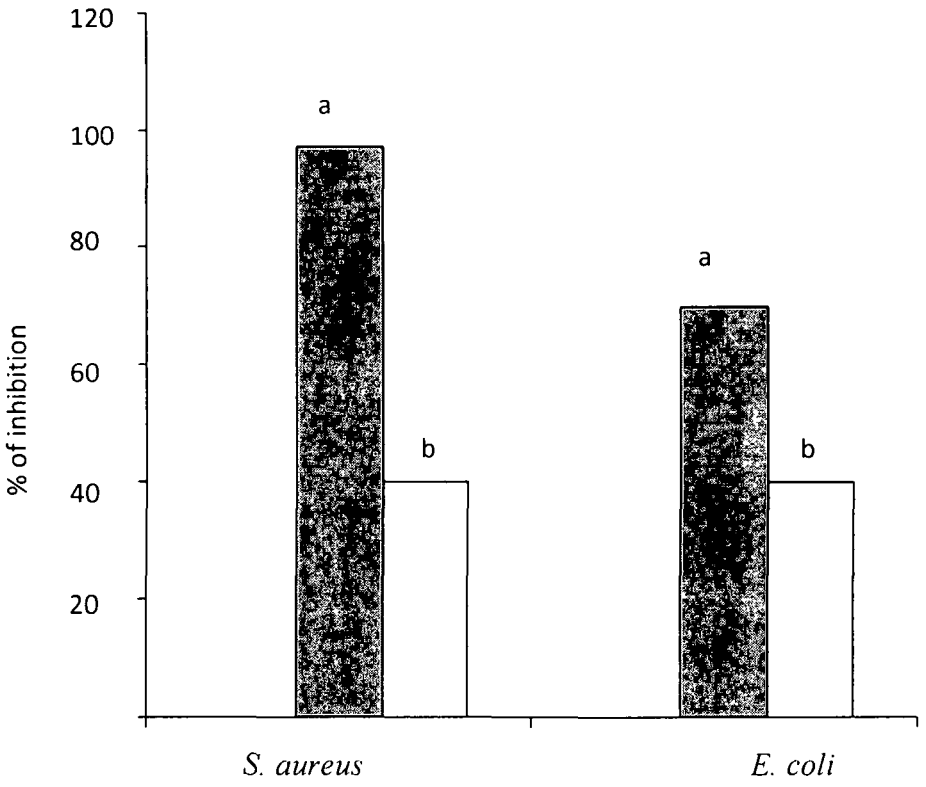

Fig. 5.7 Percent inhibition of whole cell (a) and sphaeroplast (b) of *S. aureus* and *E. coli* treated with **pV3** at 250 μM

5.4 DISCUSSION

The present study demonstrated that the compounds **3.1-3.4** and **4.2**, **4.2** are effective antibacterial agents against the representative gram-negative bacterium *E. coli* and gram-positive *S. aureus*. The extent of bactericidal effect was dependent on the concentration of pV compounds resulting in increased activity with increase in concentration. Marked differences were however noted in magnitude of inhibition of the growth of the two types of bacteria in presence of these compounds. On comparing the MIC value of non-polymer bound DPV with those of polymer

supported peroxovanadates in terms of their actual pV content (Table 5.1), it became apparent that free DPV was significantly less active than **PAV, PMAV, PSSV** and **PSS-co-MV** as well as the compound **PV3** in the inhibition of bacterial growth. The trend emerging out of our investigation on antibacterial activity of monomeric as well as polymer anchored compounds shows that bactericidal efficiency decreases in the order, **PSSV> PSS-co-MV> PMAV> PAV> pV3 > DPV > pV4**. It is therefore evident that among the tested species the polymer bound pV compounds are the most active antibacterial agents. It may thus be inferred that anchoring of pV species to appropriate polymer matrices enhances the antibacterial properties of the compounds. The greater inhibitory effect of the macromolecular pV compounds in comparison to free DPV is probably because of slower degradation of polymer bound pV as evident from their activity with catalase.

The variation in antibacterial property of the polymeric compounds as well as the difference in the inhibitor potency of the compounds towards the two types of bacteria may be attributed to the structural differences of the compounds which are likely to influence their bioavailability, as well as the difference in the outer envelope characteristics of the two bacteria[34]. The role of cell wall as a barrier to the penetration of molecules differs significantly between gram-negative and gram-positive bacteria. The outer membrane of gram-negative bacteria provides greater resistance to penetration of hydrophilic molecules compared to gram-positive bacteria which possess a permeable cell wall. This probably accounts for the consistently observed greater sensitivity of *S. aureus* to the tested compounds compared to gram-negative *E. Coli*. The ability to penetrate through or interact with bacterial cell wall are likely to differ from compound to compound due to various factors such as nature

of ligand, type of the polymeric back bone, the pendant functional groups, difference in ionic charge distribution as well as the polarity of the compounds.

The marked difference in sensitivity of the two bacteria for all the compounds tested as observed from the data could also probably be related to the localisation and nature of enzymes involved in handling oxygen radical attack. While periplasmic peroxidase or cytoplasmic superdioxide dismutase are more active in handling oxidative stress in *E .coli* and other gram negative organisms, the enzyme responsible for gram-positive bacteria are cytoplasmic superoxide dismutase along with or without extracellular catalase production. Since no difference in the sensitivity of the sphaeroplast of the two bacteria to peroxovandate moiety was found, this lends further support to the contention that accessibility of the enzyme systems differs in the two bacteria as function of their cell wall characteristics influencing the susceptibility of the organism towards the compounds.

An additional remarkable observation was that **PMAV** showed more than 50% inhibition of *S. aureus* at concentration as low as 20 μg/ml (containing 3.67 μg equivalent of pV), whereas nearly ten times higher concentration of 200 μg/ml of **PAV** was found to result in comparable inhibition of the bacterium. The higher sensitivity of *S. aureus* to **PMAV** is surprising as this compound has markedly lower percent of the active peroxovanadate moieties compared to **PAV**. Also, between the sulphonic acid containing polymers treatment with PSSV resulted in nearly 75% inhibition of S.aureous at concentration 20 μg/ml (containing 2.58 μg equivalent of pV) whereas equivalent inhibition was seen at nearly six times higher concentration of > 125 μg/ml of **PSS-co-MV.** It is tempting to suggest that the greater inhibitory effect of these compounds may again be a consequence of the notable common

feature shared by the two compounds, **PMAV** and **PSSV** which is the presence of pV units bonded to the polymer matrix in exclusively monomeric fashion. The compound **PAV** with pV moieties occurring exclusively as dimeric species is observed to be the least potent antibacterial agent among the four complexes examined. It is worth recalling in this context that a similar trend was observed earlier in case of the inhibitory effect induced by the polymeric compounds on ALP activity. Existence of a correlation between the ALP inhibitory effect of the compounds and their antibacterial activity appears possible from the aforementioned observations. However, more investigations are required to explain the findings and in absence of direct evidence at this stage we refrain from drawing any conclusion regarding definitive cause of the antibacterial effect of the title compounds.

In summary, the results demonstrate that diperoxovanadate compounds exhibit are effective antibacterial agent against gram positive *S. aureus* and gram negative *E. Coli* bacteria. It is also evident that anchoring of pV to an appropriate water soluble polymer augments its bactericidal efficiency. The results suggest the possibility of modulating the bioactivity of a pV compound by modifying the coordination environment, providing with a scope to design antibacterial agents more active against a group of bacteria.

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CHAPTER 6

Polymer anchored peroxovanadium(V) complexes mediate mild oxidative bromination*

6.1 INTRODUCTION

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

Vanadium Bromoperoxidases (V-BPO), the enzyme involved in the biohalogenation of a variety of marine natural products ranging from simple hydrocarbons to halogenated terpenes, indoles, phenols, catalyze bromination by using H_2O_2 and bromide salts[12,13]. The enzyme functions explicitly in catalyzing rate determining bromide oxidation to generate an oxidized bromine species capable of transferring bromine atoms to acceptor molecules with electron rich π bonds[14]. The oxidized bromine intermediate is likely to be equivalent of hypobromous acid (HOBr), bromine (Br_2), tribromide (Br_3^-), or an enzyme-trapped bromonium ion[12,13] although, its exact speciation is still a matter of speculation.

Taking cues from the knowledge of activity of V-BPO there have been continued efforts to develop alternative bromination protocols[13-19] and to generate environmentally benign catalytic systems for synthesis of brominated organics[8,15]. The credit for first biomimetic functional model of bromoperoxidase goes to Sakurai and Tsuchia[20]. They found

Results described in this chapter have been published in:
React. Funct. Polym., 2008, 68, 876

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[18] 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[19]. In recent years several vanadium complexes of multidentate ligands containing O and N donor sites and oxo-perox complexes V(V) have been subsequently synthesized in the quest of functional models for this enzyme and were tested for catalysis of bromide oxidation in presence of hydrogen peroxide[16 18.23]. However, contrary to natural V-BPO, which is most efficient at pH 5.5-7, several model complexes were found to be catalytically active in acid medium which limits their utility as effective catalyst[1]

It is worth mentioning in this context that success has been achieved by other workers of the laboratory where the present work has been carried out in developing peroxo complexes of V(V) and W(VI) with the ability to mediate oxidative bromination under mild condition. A series of dinuclear pV compounds with the distinctive feature of having a μ-peroxo group of the type $[V_2O_2(O_2)_3(L)_3].H_2O$[24-26] (L = asn, gln, gly-gly, gly-ala or gly-asn) could instantaneously oxidize bromide to a bromination competent intermediate in phosphate buffer at near neutral pH, also efficiently mediated bromination of organic substrates in aqueous-organic media, thus mimicking the enzyme V-BPO[24-26]. The μ-peroxo V compounds however, undergo rapid degradation in solution with loss of its high bromination activity. Nevertheless, the observation allowed to foresee potential of these compounds in biomimetic oxidative bromination provided it can be prepared in a stable form. Accordingly, we have focused anchoring pV to polymer supports anticipating that a macroligand environment will

add advantages of polymeric reagent such as stability and enhanced oxidant activity to the potential ability of the active pV species. We were particularly interested to examine whether such polymeric pV compounds could act as oxidant of bromide with good activity at neutral pH, an essential requirement of a biomimetic model. Although a few polymeric reagents are available as relatively safer stoichiometric halogenating agents, however their preparation require specific polymer backbone and direct contact with bromine[27,28].

Reported in this chapter are the results of investigations on the reactivity of the soluble polymer anchored peroxovanadates *viz.* $[V_2O_2(O_2)_4$(carboxylate)]-PA [PA = poly(acrylate)] **(PAV)[3.1]** and $[VO(O_2)_2$(carboxylate)]-PMA [PMA = poly(methacrylate)] **(PMAV)[3.2]**, $[VO(O_2)_2$(sulfonate)]-PSS [PSS = Poly(sodium 4-styrene sulfonate)] **(PSSV)[3.3]** and $[V_2O_2(O_2)_4$(carboxylate)$VO(O_2)_2$(sulfonate)]-P(SS-*co*-M) [P(SS-*co*-M)= poly(sodium styrene sulfonate-*co*-maleate)] **(PSS-co-MV)[3.4]** in oxidative bromination.

6.2 EXPERIMENTAL SECTION

6.2.1 Bromination of organic substrates and product analysis

In a representative procedure, organic substrate (0.5 mmol) was added to a solution of acetonitrile : water (1:1, 5 ml) containing Et_4NBr (2 mmol). A weighed amount of solid polymeric compound maintaining substrate : pV at 1:1, was then added to the reaction mixture at room temperature under continuous stirring. Progress of the reaction was monitored by TLC. The stirring was continued for *ca.* 7-10 h. After completion of the reaction the 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. ^1H NMR spectroscopy and melting point determinations were made to interpret the products.

6.2.2 Regeneration of the oxidant

The regeneration of the compound **PAV [3.1]** and **PSS-co-MV [3.4]** for reuse was tested for the reaction using p-nitroaniline as substrate. The reaction mixture contained the same recipe mentioned above with a 4 fold increase in the amounts of the components. In order to regenerate **PAV [3.1]** after completion of the reaction, the following method has been adopted. After extraction of the organic reaction product, the aqueous part of the reaction mixture was transferred to a 250 ml beaker. Keeping the solution in an ice bath, 2 ml H_2O_2 was added to it maintaining the V: peroxide at 1:4 followed by addition of excess DMF with constant stirring until a yellow coloured mass separated out. After allowing it to stand for 5 min in the ice bath, the supernatant liquid was decanted off and the residue was treated repeatedly with DMF and distilled acetone until it became yellow microcrystalline solid. The product was separated by centrifugation, washed with cold acetone and dried *in vacuo* over concentrated sulfuric acid. Using same strategy the regeneration of **PSS-co-MV[3.4]** was also accomplished after the completion of the reaction.

6.2.3 Measurement of Bromination activity in solution

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 a bromophenol blue (ε^{592} mM = 67.4) was used to measure bromination activity. The reaction mixture contained phosphate buffer (50 mM, pH 5.5), KBr (1 M) and phenol red (20 μM). The redox activity was tested by adding a measured amount of aliquot from complex solution and by monitoring the possible change in the absorbance at 592 nm at 30 ^{0}C. The volume of the reaction mixture was kept at 25 ml. Aliquots were transferred to the spectrophotometer immediately after mixing.

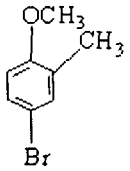
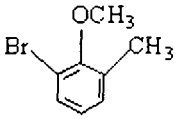
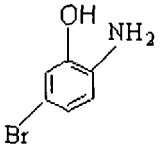
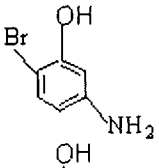
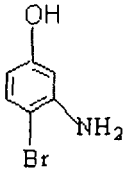
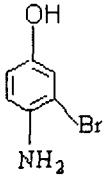
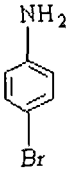
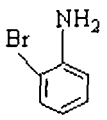
6.3 RESULTS AND INTERPRETATION

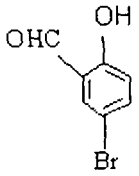
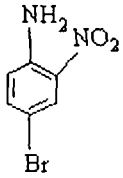
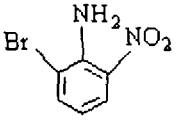
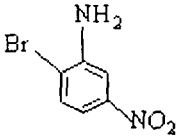
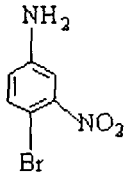
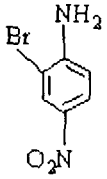
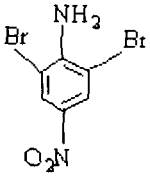
6.3.1 Substrate bromination in aqueous-organic media

Efficacy of each of pV complexes in mediating bromination of organic substrates in presence of bromide in aqueous-organic media has been explored. Brominations of several activated aromatics into their corresponding bromo-organics were achieved simply by stirring a solution of the substrate in presence of **PAV[3.1]** and **PSS-co-MV[3.4]** in CH_3CN:H_2O (1:1) for 7-10 h at ambient temperature.Results obtained by using **PAV** as a representative are presented in Table 6.1. Tetraethyl ammonium bromide (Et_4NBr) was used as source of bromide. The condition of reactions such as reaction temperature substrate: oxidant stoichiometry, bromide concentration and type of solvent were optimized using the substrate p-nitroaniline as a representative. A 1:1 oxidant: substrate stoichiometry appeared to be optimal and the solvent CH_3CN : H_2O (1:1) provided good yields of the products. Activated aromatics such as aniline and acetanilide were brominated to produce predominantly p-bromo products. The remarkable feature of the present methodology is that the reaction takes place at near neutral pH and no extra addition of acid or H_2O_2 is required for the stoichiometric bromination reaction of the substrate. It was intriguing to note that under the optimized conditions the neat DPV complex was totally inactive in bromination and **PMAV[3.2]** and **PSSV[3.3]** showed poor oxidant activity affording the brominated products in <5% yield.

Preferential bromination at either ortho or para position of the aromatic ring leading to mono substitution indicates an electrophilic bromination mechanism. That the brominating species was 'Br^+' and not a Br radical in these reactions was further evident from the ring substituted products obtained from the substrate, 2-methoxytoluene (Fig 6.1). Bromination through radical reaction would have produced benzyl bromide instead of bromo-methoxy toluene.

Table 6.1. Bromination of organic substrates mediated by **3.1**

Substrate	Product	Chemical Shift δ ppm	% Yield	Time(hr)
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	60	8
		7 7-7 4(m, 3H), 3 64(s, 3H, -OCH_3), 1 1 (s, 3H, -CH_3)	25	
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)	83	8
m-Aminophenol		7 8-7 25 (m, 3H), 5 3-5 0 (br, 2H, -NH_2)	77	8
		8 1-7 5 (m, 3H), 5 6-5 3 (br, 2H, -NH_2)	18	8
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)	85	8
Aniline		7 16 (d, 2H, J=7Hz), 6 5 (d, 2H, J=7Hz), 3 5 (s, 2H, -NH_2)	80	8
		7 5-6 36 (m, 4H), 4 0 (s, 2H, -NH_2)	15	

Substrate	Product	Chemical Shift δ ppm	% Yield	Time(hr)
Salicylaldehyde		10 0 (s,CHO), 5 (-OH)	80	10
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)	70	7
		7 3-7 05 (m, 3H), 6 5 (s, 2H, -NH_2)	23	
m-Nitroaniline		7 7-7 25 (m, 3H), 4 6-5 0 (br, 2H, -NH_2)	65	7
		7 5-6 7 (m, 3H), 4 5-4 8 (br, 2H, -NH_2)	30	
p-Nitroaniline		8 25 (s, 1H), 7 9(d, 1H, J=6Hz), 6 64 (d, 1H, J=6Hz), 4 8-4 6 (br, 2H, -NH_2)	75	7
		8 05 (s, 1H), 4 0-4 . -NH_2 =6Hz)	21	

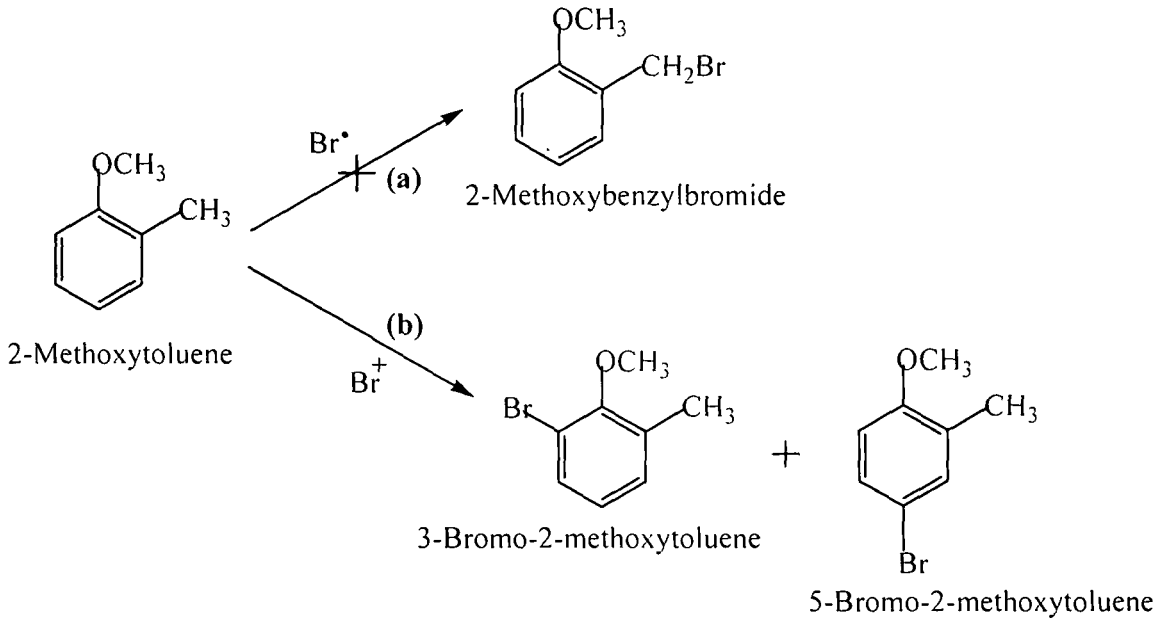


Fig. 6.1 Bromination reaction of 2-methoxytoluene. (a) Possible product of bromination through radical reaction, (b) electrophilic bromination involving 'Br^{+}' forms exclusively ring substituted products. Bromination reaction using peroxovanadium compound **3.1** produces exclusively bromomethoxytoluene.

6.3.2 Regeneration of the reagent

Most frequently, recovery of the soluble polymer supported reagent can be achieved by diluting the homogeneous polymer solution by the addition of an excess of a poor solvent which induces precipitation of polymeric species[30,31]. The resulting heterogeneous mixture is filtered to obtain the polymer. For satisfactory recovery of a soluble polymer from reaction mixture proper choice of solvent and temperature are crucial.

In the present study regeneration of the oxidant **PAV[3.1]** or **PSS-co-MV[3.4]**was accomplished easily by treating the aqueous extract of the spent reaction mixture with H_2O_2, maintaining the peroxide: V ratio at 1:4, followed by the addition of DMF to this solution at

ambient temperature which induced complete precipitation of the polymer-bound complex. Since Et_4NBr used in excess as a bromide source in the reaction is soluble in DMF, possibility of co-precipitation of this species along with the polymer could be ruled out. Addition of hydrogen peroxide was necessary in order to compensate the peroxide consumed during the bromination reaction. The IR spectrum of the recovered product was identical with the original starting complex which showed the presence of side-on bound peroxo group and bridged carboxylate group when PAV was used as oxidant. Reuse of the recovered complex in a fresh cycle of bromination reaction afforded the desired brominated products indicating that metal complexes are intact with the polymer chain and the oxidant is active even after the first cycle. However, a slight decrease in product yield obtained suggested the possibility of some leaching of the metal over subsequent reuse of the compound.

6.3.3 Activity of the complexes in bromination in aqueous solution

With an objective to gain a better understanding of the reactivity of the compound **PAV[3.1]** and **PSS-co-MV** in mediating oxidative bromination, we considered it imperative to further investigate the reaction in solution in line with our earlier work on bromination activity of peroxovanadates[24,25,32]. The bromination of phenol red to its tetra brominated product, bromophenol blue was used to measure the bromination activity of the complex **PAV[3.1]** in solution. Addition of freshly prepared aqueous solution of each of the complexes **PAV** and **PSS-co-MV** at concentration indicated (Table 6.2) to the standard reaction of bromide in phosphate buffer with phenol red as trap for oxidized bromine resulted in gradual color change of the solution from yellow to blue[29]. 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 2*). The data in the Table 6.2 show that the complxes **3.1** and **3.4** posses bromination activity with a initial rate of bromine transfer 3.1

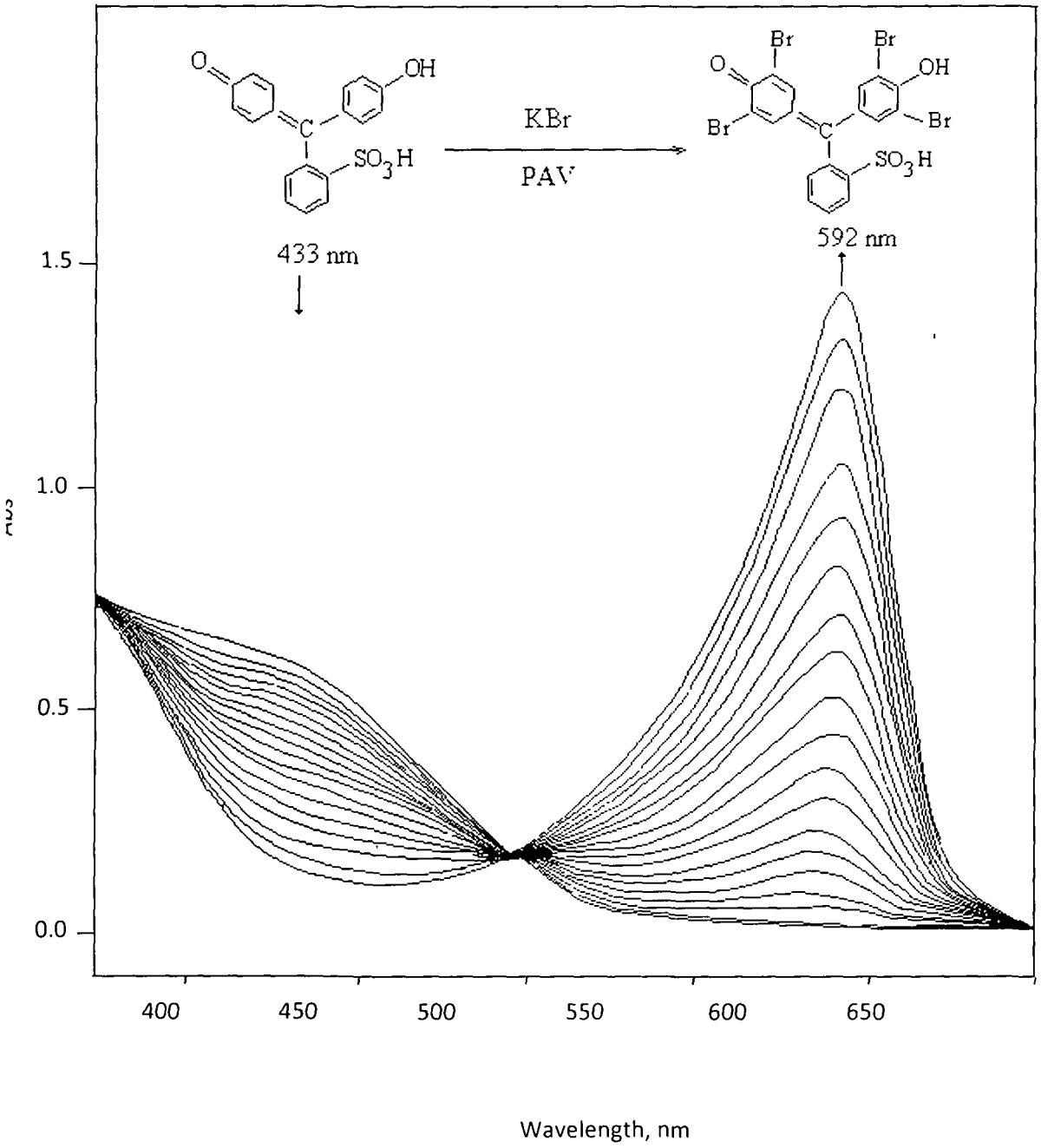


Fig. 6.2 Bromination activity with **PAV**. Spectral changes at 2 min interval following bromination of phenol red to bromophenol blue on addition of compound solution to the reaction mixture containing phosphate buffer (0.05 M, pH 5.5), KBr (1 M) and phenol red (20 μM) and PAV (0.11 mg/ml).

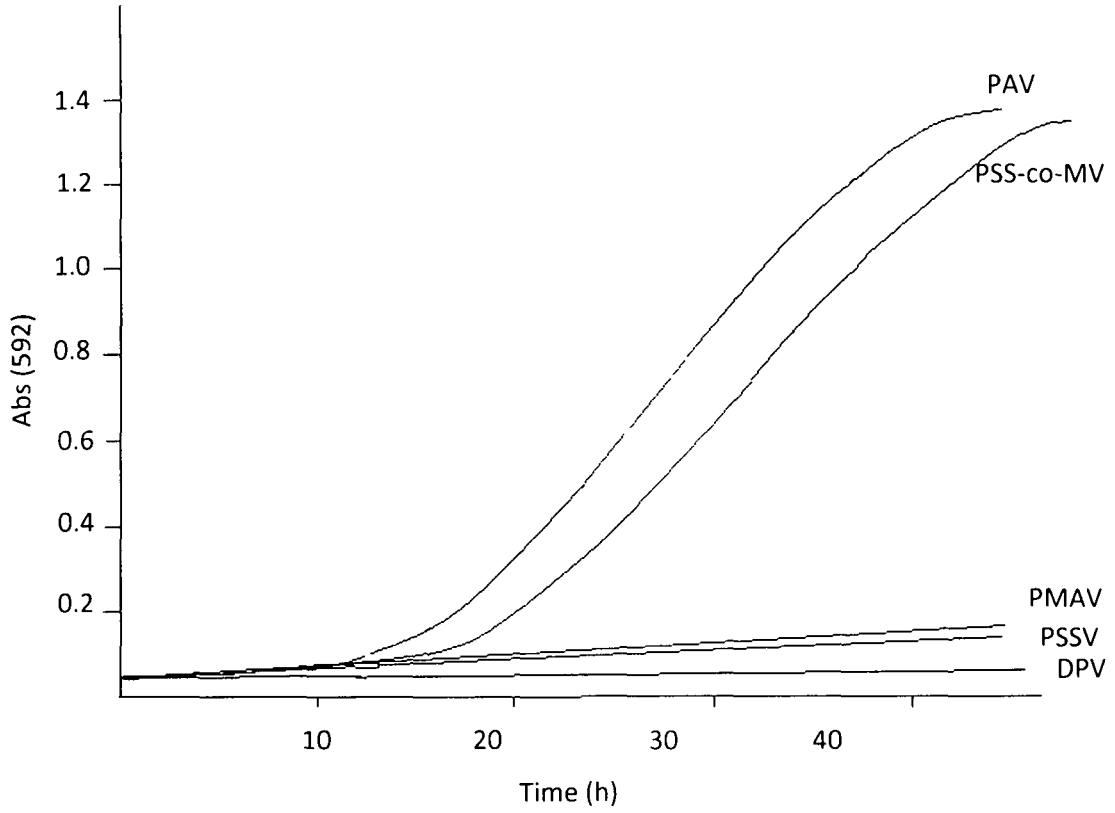


Fig. 6.3 The increase of A_{592} for bromophenol blue indicating the rate of bromination of phenol red with PAV. The reaction mixture contained phosphate buffer (0.05 M, pH 5.5), KBr (1 M) and phenol red (20 μM) and **PAV** (0.11 mg/ml) or **PSS-co-MV**(0.19 mg/ml)

the peak at A_{433} due to loss of phenol red (*Fig 6 2*). The data in the Table 6.2 show that the complxes **3.1** and **3.4** posses bromination activity with a initial rate of bromine transfer 3.1 μMBr/min for **PAV** and 2.7 μMBr/min for **PSS-co-MV** (Table 6.2). The constant rate of the reaction during which A_{592} progressively increased was preceded by an initial lag. It was of interest to note that the bromination activity shown by **PMAV[3.2]** and **PSSV [3.3]**was practically negligible and DPV as such was totally inactive in bromination (*Fig 6.2*).

Table 6.2 *Bromination of phenol red with peroxovanadate complexes* **3.1-3.4**

No.	Compounds	Conc(mM) in terms of pV loading	Rate of bromine transfer	
			ΔA_{592}/min	μM Br/min
1.	$[V_2O_2(O_2)_4$(carboxylate)]-PA(**PAV**)(**3.1**)	0.2	0.052	3.1
2.	$[V_2O_2(O_2)_4$(carboxylate)$VO(O_2)_2$(sulfonate)] -P(SS-*co*-M) **PSS-*co*-MV(3.4)**	0.2	0.045	2.7
3.	$[VO(O_2)_2$(carboxylate)]-PMA(**PMAV**) (**3.2**)	0.2	-	-
4.	$[VO(O_2)_2$(sulfonate)]-PSS **PSSV (3.3)**	0.2	-	-

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 solution of compound **PAV[3.1]**. Addition of phenol red to this solution resulted in the decrease in A_{262} nm and a peak at 592 nm appeared indicating the formation of bromophenol blue. The 262 nm peak, therefore, represents a bromination competent oxidized species of bromide, probably an equilibrium mixture of BrOH, Br_2 and Br_3^- as proposed earlier.

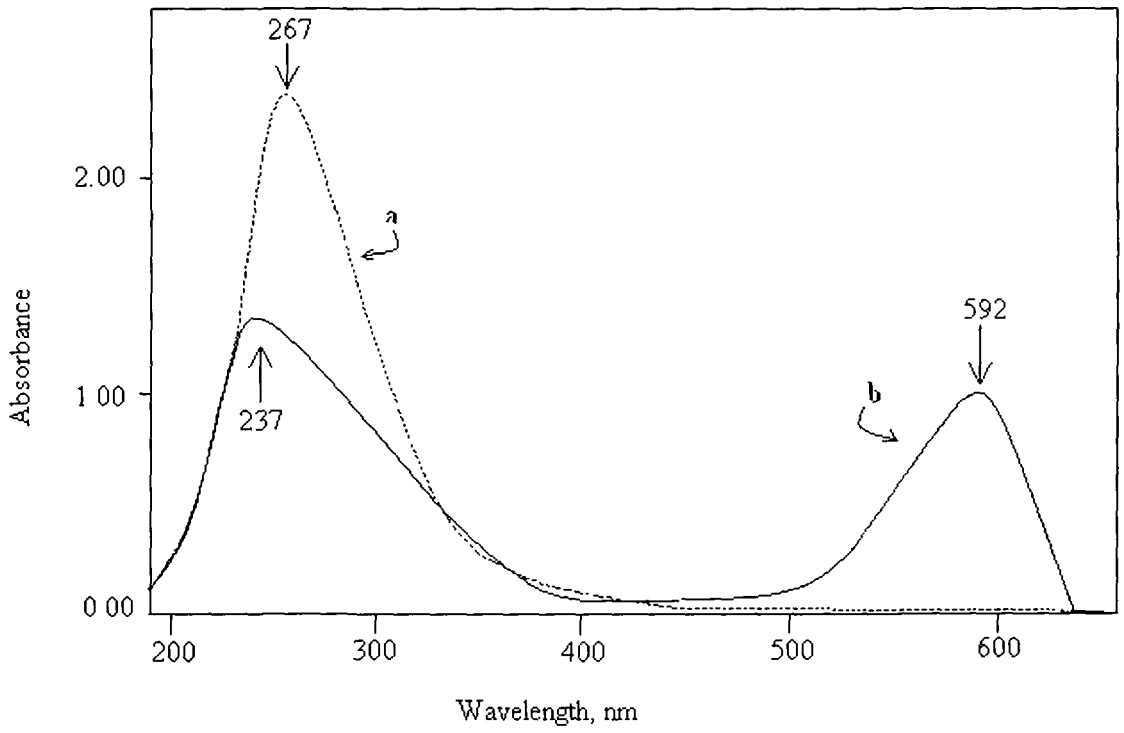


Fig. 6.4 Spectral changes following bromination of phenol red to bromophenol blue on addition of PAV. The reaction mixture contained phosphate buffer (0.05 M, pH 5.5), KBr (1 M) and phenol red (20 μM) PAV (0.11 mg/ml).(a) KBr + compound in absence of phenol red (b) KBr + compound + phenol red.

6.3.4 Effect of H_2O_2 on peroxovanadate mediated bromination

The effect of H_2O_2 on the bromination activity under the standard assay condition was tested. While the initial addition of H_2O_2 (0.5 mM) to the reaction solution had no observable effect on the initial rate of bromination, a revival of the bromination activity was noted on addition of H_2O_2 (0.5 mM) to the spent reaction mixture which contained excess bromide and substrate. The reaction thus could be made catalytic by the addition of exogenous hydrogen peroxide which is apparently required for *in situ* regeneration of the active brominating species.

6.3.5 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[33]. Vanadate is known to react with most of the compounds used in buffers including phosphate[34]. With respect to vanadates Hepes is fairly inert and is the recommended buffer[35],[36] However, in the present study the observed bromination activity was found to be suppressed when Hepes (pH 6.5) was used as buffer indicating possible interaction between Hepes and the 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[35.36]

6.4 DISCUSSION

The work of several groups on redox chemistry of vanadate and peroxovanadates [33-39] are pertinent in explaining the observed activity of the polymer bound dimeric tetraperoxovanadate compound **PAV[3.1]** and **PSS-co-MV[3.4]** in oxidative bromination and inactivity of **PMAV[3.2]** and **PSSV[3.3]** in the same reaction under analogous condition. It has been known that oxidation of bromide by H_2O_2 to hypobromous acid, capable of transferring a bromine atom to an acceptor molecule, occurs at a pH< 3.0 but is ineffective in near neutral pH >5.0. The reaction is catalyzed by vanadium compounds such as $VOSO_4$[20] or V_2O_5[19]. The most probable oxidants of bromide, monoperoxovanadate, $VO(O_2)^+$ (MPV), and diperoxovanadate $VO(O_2)_2^-$ (DPV), are readily formed on adding excess H_2O_2 to vanadate solution , with DPV predominating at pH> 5.0. However, synthetic DPV or monoperoxovanadate of and or compounds containing such moieties were catalytically incompetent in bromide oxidation at neutral pH[41]. The observation that inactive DPV requires the presence of vanadyl or vanadate to gain oxidant activity at pH>5, led to the proposal that μ-peroxo-bridged divanadate intermediate, $[OVOOV(O_2)]^{3+}$, formed by complexation between these two species is the proximate oxidant of bromide[36]. Support for such an intermediate came from our investigations on a series of synthetic compounds with a VOOV bridge which were found to be active in oxidative bromination at neutral pH[244,255]. Based on these findings it was suggested earlier that a μ-peroxovanadate group may be the principal requirement for bromide oxidation by pV compounds at neutral pH[24,332]. It was further shown that monomeric diperoxovanadate species, containing exclusively side-on bound peroxo groups were inefficient in bromination at neutral pH[244].

Taking the above observations into account it is reasonable to expect that anchoring of the pV in the form of a dimer to the polymeric chain where two **pV** units are held together in close proximity by a bridging carboxylate, as observed in case of the complexes **PAV[3.1]** and to some extent in **PSS-co-MV[3.4]**, would facilitate the formation of a peroxobridged species which then can act as bromide oxidant. The initial lag observed in the rate of bromination of phenol red mediated by the polymeric compounds, which is in contrast to the instantaneous bromination of the substrate brought about by the unbound dinuclear Pv complexes with a μ-peroxo group[24,25], may be interpreted in terms of the time required for the formation of the peroxo-bridged intermediate in solution. In the compounds, **PMAV[3.2]** and **PSSV[3.3]** with relatively lower V loading and **pV** units distributed over the polymer matrix as monomeric DPV, such bridge formation is unlikely which probably explains its inactivity in bromination. Thus the findings of the present study are in accord with the earlier suggestion that for a **pV** complex to be active in bromination at neutral pH a μ-peroxovanadate configuration is the pre-requisite.

In summary the newly synthesized **PAV[3.1]** and **PSS-co-MV[3.4]** complex could serve as stoichiometric reagent for bromide oxidation in aquous-organic medium at neutral pH, whereas **PMAV[3.2]** and **PSSV[3.3]** are inactive in bromination. In view of the redundancy of bromine or hydrobromic acid in the bromination process mediated by it, and environmentally acceptable reaction condition, we may expect **PAV[3.1]** and **PSS-co-MV[3.4],** to emerge as a useful addition to the range of biomimetic synthetic oxidants in bromide oxidation. The result of the investigation is consistent with the proposal implicating formation of a peroxo-bridged divanadate intermediate which is active in bromination. Further information in support of this aspect, generated from our theoretical investigation on structure and reactivity of a dinuclear μ-peroxovanadate complex, is being presented in the next chapter.

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CHAPTER 7

Density functional studies on structure and reactivity of a dinuclear peroxovanadate(V) complex

7.1 INTRODUCTION

It is evident from the available literature that exact mechanism involved in the function of bromoperoxidase and the actual role of vanadium in these processes are yet to be fully understood despite the progress made in recent years[1-8]. Studies on synthetic peroxovanadate (**pV**) complexes as functional and structural models of bromoperoxidase (V-BPO) 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[6-11]. The H_2O_2-dependent oxidation of bromide is a two-electron transfer both in the enzyme reaction at pH 6.5 [9] and in the chemical reaction with cis-dioxovanadium in highly acidic medium[10]. Vanadium atoms appear to aid the overall catalytic process by presenting a modified peroxide species as the oxidant at physiological pH[11-14]. The biomimetic models of bromoperoxidation reaction therefore proposed vanadium derivatives such as peroxovanadates [15,16] and their oxo or peroxo-bridged dimers as the oxidants[3,17,18].

The structurally characterized synthetic hetero-ligand peroxovanadate(V) complexes mostly represent anionic mono, di or tetra peroxo complexes containing peroxo group bonded to vanadium in a side-on fashion[4]. Reports related to dinuclear peroxovanadates with bridging peroxo group are very limited probably due to the

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difficulties encountered in stabilizing and isolation of such complexes[19-25]. Such pV species have been implicated as intermediates in some biochemical processes including bromide oxidation, oxidation of NADH[26], inactivation of glucose oxidase[27], and hydroxylation of benzoate[28]. Oxygen is released in absence of an oxidisable substrate[28]. It has already been mentioned in Chapter 6 that a number of triperoxo divanadate complexes with a bridged peroxo group and amino acids and small peptides serving as ancillary ligands, synthesized in recent years in our laboratory[22,24] distinguished themselves in terms of their spectral as well as solution properties from the known peroxo-vanadium complexes possessing exclusively side-on bound peroxo groups[22,24]. The complexes exhibited unique redox properties and could act as efficient bromide oxidant at physiological pH[22-24,29]. This has been a noteworthy observation since most of the synthetic peroxovanadate compounds studied as functional model of the BrPO are active only at acidic pH[1,5,6,8] The information 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[18]. The proposed reaction pathway based on experiments undertaken in our laboratory and work of some other researchers conferred the status of a selective bromide oxidant, at physiological pH, on VOOV group[18,22,24]. The μ-peroxo group of the VOOV moiety appears to be amenable for reductive cleavage by bromide which produces a bromination competent intermediate, postulated to be -BrOVO(O_2), that can transfer the bromine atom to the substrate[18,22-24,30]. Results of previous investigations indicated an electrophilic bromination mechanism. However, the cause of this greater reactivity of a peroxo-bridged divanadate moiety compared to a η2 -peroxo group, in an oxidative bromination

reaction was not completely clear. The crystal structures of these complexes could not be resolved so far due to their unstable nature in solution[22-24]. It is also interesting and relevant in this context that the newly synthesized polymer anchored compounds where **pV** moieties were anchored as isolated DPV units were observed to be inactive in mediating bromination whereas compounds with dimeric **pV** moieties were active in such reactions.

As a sequel of studies on the afore mentioned aspects of peroxovanadates and as part of a general programme of the laboratory, it was considered imperative to investigate the structural and electronic properties of dinuclear peroxovanadates using quantum chemical methods. Modern computational chemistry is increasingly being recognized as an important tool to investigate structural and electronic properties of metal complexes[31]. In recent years, density function based reactivity descriptors have been found to be quite successful in analyzing chemical reactivity and selectivity[32-35]. While global quantities like electronegativity (χ), hardness (η), softness (S), electrophilicity index (ω) take care of the reactivity of molecules, the selectivity of each atomic site in a molecule is characterized by the local quantities like Fukui function, local softness, philicity, relative electronegativity and relative nucleophilicity[32]. To the best of our knowledge there has been no available reports on quantum chemical studies on structure and properties of peroxo-bridged vanadium complexes, although theoretical investigations on structure and electronic properties of vanadium complexes have been carried out earlier[36,37].

In the present work the structural and electronic properties of a dinuclear peroxo-bridged vanadium complex $[V_2O_2(O_2)_3(glycine)_2]$ have been investigated using density functional theory. The initial structure of the complex has been modeled from the

available spectroscopic and chemical analysis data related to the synthetic compound $[V_2O_2(O_2)_3(glycine)_2(H_2O)_2].H_2O$[38]. The structure has been optimized and the reactivity of selected atoms was determined by calculating their Fukui function and relative electrophilicity. Attempt has been made to correlate the results of the study to the observed reactivity pattern of the complex species in oxidative bromination.

7.2 Theoretical Background

The global quantities electronegativity (χ) and hardness (η), for an N-electron system with total energy E are defined as[39]:

$$\chi = -\mu = -\left(\frac{\partial E}{\partial N}\right)_{v(\vec{r})} \qquad (1)$$

$$\eta = \frac{1}{2}\left(\frac{\partial^2 E}{\partial N^2}\right)_{v(\vec{r})} = \frac{1}{2}\left(\frac{\partial \mu}{\partial N}\right)_{v(\vec{r})} \qquad (2)$$

where μ is chemical potential of electron and $v(\vec{r})$ is external potential. The global softness (S) is the inverse of hardness with a factor half.

$$S = \frac{1}{2\eta} = \left(\frac{\partial N}{\partial V}\right)_{v(\vec{r})} \qquad (3)$$

The local quantities are important in determining the reactivity and selectivity of a specific atom or group in a molecule.

The Fukui function which has been found to be an important local quantity is defined as[40]

$$f(\vec{r}) = \left(\frac{\partial \rho(\vec{r})}{\partial N}\right)_{v(\vec{r})} = \left(\frac{\partial \mu}{\partial v(\vec{r})}\right)_{N(\vec{r})} \qquad (4)$$

Owing to the discontinuities in the plot of $\rho(\vec{r})$ versus N, there exist three different types of Fukui functions namely,

$f^{+}(\vec{r}) = \rho_{N+1}(\vec{r}) - \rho_{N}(\vec{r})$ for nucleophilic attack on the system (5a)

$f^{-}(\vec{r}) = \rho_{N}(\vec{r}) - \rho_{N-1}(\vec{r})$ for electrophilic attack on the system (5b)

$f^{0}(\vec{r}) = \frac{1}{2}[\rho_{N+1}(\vec{r}) - \rho_{N-1}(\vec{r})$ for radical attack on the system (5c)

where $\rho_{N}(\vec{r})$ is the electron density of the N-electron ($N \equiv N-1$, N, $N+1$) species. Substituting these electron densities by respective electron polulations (q_k) on the atomic site k of the molecule one can calculate the corresponding condensed Fukui functions.

Roy et al[41] introduced another two reactivity parameters which are known as relative electrophilicity and relative nucleophilicity. These two reactivity parameters can be calculated as below:

Relative electrophilicity = $\frac{f_k^{+}}{f_k^{-}}$ and Relative nucleophilicity = $\frac{f_k^{-}}{f_k^{+}}$

7.3 Computational Details

We generated the initial structure of the peroxo-bridged vanadium complex $[V_2O_2(O_2)_3(glyH)_2]$ from the information gained through spectroscopic and chemical analyses data related to the synthetic compound $[V_2O_2(O_2)_3(glycine)_2(H_2O)_2]$. We use the DMol3 program in our calculations[42]. The DNP numerical basis set used in our calculations is the highest quality set available in DMol3. This set contains a polarization *d* function on heavy atoms and a polarization *p* function on hydrogen. It is comparable in

size to the 6-31G** basis set however, numerical basis sets of a given size are much more accurate than Gaussian basis sets of the same size. We calculated bond length and vibrational frequencies of O-O, and V-O bonds and compared them with available experimental and theoretical results. We also calculated the Fukui function and relative nucleophilicity values using Mulliken and Hirshfeld population schemes to demonstrate reactivity of O-O moieties.

7.4 RESULTS AND INTERPRETATION

7.4.1 Spectral Properties

With an objective to gain an insight into the afore mentioned aspects, in the present work we investigated the structural and electronic properties of a dinuclear peroxo bridged vanadium complex $[V_2O_2(O_2)_3(glycine)_2]$ using density functional methods. The initial structure of the complex has been modelled from the available spectroscopic and chemical analysis data related to the synthetic compound $[V_2O_2(O_2)_3(glycine)_2(H_2O)_2]$ [38]. The structure has been optimized with PW91 and BP functionals without imposing symmetry constraints using the DMol3 program[42]. In fact we generated several initial structure of the peroxovanadium complex by changing the orientations of the ligands. The structure shown in Fig. 7.1 converged to a minimum with no negative vibrational frequency. The calculated bond lengths and vibrational

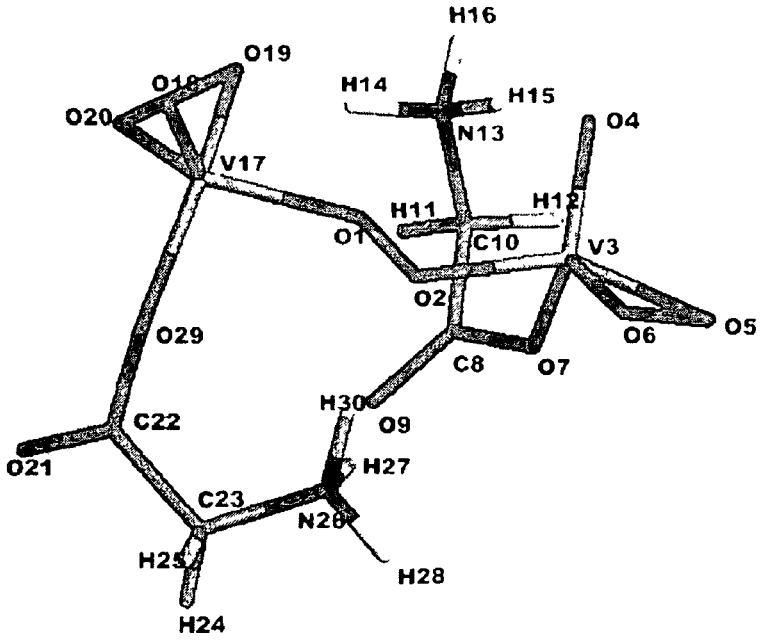

Fig. 7.1 View of $[V_2O_2(O_2)_3(glycine)_2]$ complex showing 6 hydrogen bonds in the optimized geometry derived from PW91/DNP method. The pairs which form hydrogen bonds are: O_4----H_{15}, O_9----H_{27}, O_{19}----H_{14}, O_2----H_{30}, O_1----H_{14} and O_1----H_{30}. Oxygen atoms O_1—O_2 form the bridge peroxide group whereas oxygen atoms O_5—O_6 and O_{19}—O_{20} form the two terminal peroxide groups.

frequencies of O-O, and V-O bonds have been compared with the available experimental and theoretical results. We have also calculated the Fukui function and relative electrophilicity values using Mulliken and Hirshfeld population schemes in order to understand the reactivity of O-O moieties.

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_s and $\nu_{as.}$ which involve metal-oxygen stretches, appearing in the region 500–600 cm^{-1} [43,44]. In the spectra of the bridged peroxovanadate complexes in addition to the strong ν(O—O) absorption occurring at *c.*835 cm^{-1}, a weak intensity but well resolved band has been observed at a lower frequency range of 805-820 cm^{-1} which has been assigned to the ν(O—O) band of the bridging peroxo group. This observation was interpreted as an indication of the presence of two structurally different peroxo groups, the edge-bound and bridging type[22-24,38]. The LR spectra of the complexes complimented their IR spectra confirming the presence of two types of peroxo groups, terminal and bridging peroxides.

The PW91/DNP calculated vibrational frequencies are compared with experimentally determined frequencies in Table 1. The average error for frequencies calculated with B3LYP functionals was reported to be of the order of 40-50 cm^{-1} for small inorganic molecules[45]. In the present case therefore, in view of the relatively large size of the molecule, the discrepancy observed between calculated and measured frequencies appears to be acceptable. Inspection of the data in Table 1 shows the presence of two kinds of O-O groups in the model complex viz, 923 and 834 cm^{-1} attributable to the edge-bound and bridging peroxo groups, respectively.

Table 7.1.

Experimental and computed IR bands of the dinuclear peroxovanadate complexes indicating the presence of two kinds of O—O linkages

Complex	Infrared (IR) bands, cm^{-1}				Ref
	$\nu_s(V—O_2)$	$\nu_{as}(V—O_2)$	$\nu(O—O)$	$\nu(V=O)$	
$V_2O_2(O_2)_3$ (gly-gly)$_3$.H_2O	561 m	613 m	835 s	958 s	14
			803 w		
$V_2O_2(O_2)_3$ (gly-ala)$_3$.H_2O	572 m	620 m	835 m	949 s	14
			803 w		
$V_2O_2(O_2)_3$ (gly-asn)$_3$.H_2O	578 m	642 m	815 m	930 s	14
$V_2O_2(O_2)_3$ (glyH)$_2$ (H_2O)$_2$	530 m	605 m	835 m	959 s	13
			820 w		
	PW91/DNP Computed IR bands, cm^{-1}				
$V_2O_2(O_2)_3$(glyH)$_2$	566 m		923 s	983s	
			834 w		

7.4.2 Geometrical Parameter

The PW91/DNP optimized geometry of the modeled peroxo vanadate complex is being presented in Figure 1. Oxygen atoms O_1—O_2 form the bridge peroxide group whereas oxygen atoms O_5—O_6 and O_{19}—O_{20} form the two terminal peroxide groups. The selected geometrical parameters of the complex calculated at PW91/DNP and BP/DNP levels are given in Table 7.2. It is seen from Table 2 that the O_1—O_2 bond length (bridge peroxo group) is slightly longer than that of the two terminal peroxide groups. The average O—O bond lengths calculated at BP/DNP and PW91/DNP levels of calculations are 1.452 Å and 1.450 Å, respectively for the bridging and terminal peroxo groups. These values are in good agreement with the experimental O—O bond length (1.424 to 1.503

Å) for bound peroxo groups bonded in a side-on fashion[4]. The BP/DNP calculated average V—O bond length, 1.890 Å of $V\text{-}O_2^{2-}$ group and average V=O bond length, 1.617 Å are also in good agreement with the experimental values[4] of vanadium complexes where V—O and V=O bonds are present (Table 2). Similar results are obtained at PW91/DNP level of calculation.

Six hydrogen bonds involving amine hydrogens (H_{14}, H_{15}, H_{30}), peroxo oxygens (O_1, O_2, O_{19}), carbonyl oxygen (O_9) and terminal oxo group ($V{=}O_4$) are observed in the optimized structure (Figure 1). All the six hydrogen bonds present in the complex are given in Table 2. The distances between the amine hydrogen (H_{14}) and peroxo oxygen (O_{19}), amine hydrogen (H_{27}) and carbonyl oxygen (O_9), amine hydrogen (H_{15}) and terminal oxo group ($V{=}O_4$) and amine hydrogen (H_{30}) and peroxo-bridged oxygen (O_2) are 1.597, 1.690, 1.794 and 1.827 Å, respectively, at PW91/DNP level of calculation which indicate the presence of strong intramolecular interaction in the complex. The presence of the H-bonds in the compounds lend support to our earlier suggestion that the amino acid and peptide ligands impart stability to the highly reactive 'VOOV' species by inter-ligand interaction possibly hydrogen bonding, affording their isolation into solid state[18,22-24]. It is significant to note that such dimeric compounds could be isolated only in the presence of co-ligands with ability to form H-bond, such as amino acids and peptides. The dinuclear compounds although reasonably stable in the solid state undergo rapid degradation on dissolving in water with partial loss of peroxide. Ready loss of hydrogen bonds indeed is the likely cause of the instability of these compounds in water.

Table 7. 2.

Selected geometric parameter of vanadium complex calculated at BP/DNP and PW91/DNP levels.

Geometrical parameter[a]	Level of calculations		Available Experimental values[b]
	BP/DNP	PW91/DNP	
O_1—O_2	1.458	1.455	
V_3—O_2	1.918	1.915	1.810-1.999
V_3=O_4	1.635	1.634	1.574-1.625
V_3—O_5	1.813	1.810	1.810-1.999
V_3—O_6	1.865	1.862	1.810-1.999
V_3—O_7	1.967	1.959	1.810-1.999
O_5—O_6	1.445	1.442	1.424-1.503
V_{17}—O_1	1.899	1.893	1.810-1.999
V_{17}—O_{18}	1.599	1.597	1.574-1.625
V_{17}—O_{19}	1.911	1.913	1.810-1.999
V_{17}—O_{20}	1.848	1.846	1.810-1.999
V_{17}—O_{29}	1.902	1.893	1.810-1.999
O_{19}—O_{20}	1.453	1.452	1.424-1.503
Hydrogen bonding			
O_4----H_{15}	1.811	1.794	
O_9----H_{27}	1.713	1.690	
O_{19}----H_{14}	1.619	1.597	
O_2----H_{30}	1.849	1.827	
O_1----H_{14}	2.391	2.384	
O_1----H_{30}	2.378	2.360	

[a]See Fig. 1 for the atomic numbering. Bond distances in Å.

[b]From reference [4]

7.4.3 Chemical Reactivity

In order to understand why a μ-peroxo group of a dinuclear peroxovanadate complex is so strongly activated toward oxidative bromination we performed theoretical calculations to derive atomic charges and DFT based reactivity descriptors.

7.4.4 The charge distribution:

Expecting that the net atomic charges of atoms in a molecule may provide valuable information about the reactivity of various atoms we derived the atomic charges of selected atoms of the complex using Hirshfeld and Mulliken schemes (Table 3). The oxygen atoms belonging to the terminal peroxo groups are the most negatively charged (-0.16, average value) followed by the bridging peroxo group (-0.14) as derived from Hirshfeld population scheme of PW91/DNP method. The bridge peroxo group would therefore be expected to be the best candidate to interact with an electron rich site such as Br^-, according to the "electrophilic" mechanism proposed for the bromination reactions mediated by vanadium complexes[1,3,4,6,24]. However, similar trend was not observed from the atomic charges derived by Mulliken population scheme. The BP/DNP calculated average Hirshfeld charges of bridging and terminal peroxo groups are also -0.14 and -0.16 respectively. Similar to PW91/DNP calculated Mulliken charges the BP/DNP calculated Mulliken charges do not show the correct reactivity of the peroxo groups. This discrepancy may be related to the arbitrariness of the Mulliken charges definition, especially for relatively large bases.

Table 7.3.

PW91/DNP and BP/DNP calculated charges. Fukui functions and relative electrophilicity of selected atoms of the vanadium complex derived from Hirshfeld and Mulliken population schemes.

Selected atoms		Mulliken population scheme				Hirshfeld population scheme			
		q	f^-	f^+	f^+/f^-	q	f^-	f^+	f^+/f^-
O_1		-0.440	0.011	0.010	0.909	-0.155	0.015	0.018	1.200
O_2		-0.362	0.046	0.057	1.239	-0.131	0.039	0.048	1.231
O_5	PW91/	-0.278	0.114	0.114	1.000	-0.142	0.112	0.106	0.946
O_6	DNP	-0.289	0.132	0.103	0.780	-0.163	0.125	0.092	0.736
O_{19}		-0.402	0.079	0.047	0.595	-0.160	0.077	0.042	0.545
O_{20}		-0.300	0.110	0.078	0.709	-0.160	0.103	0.067	0.650
V_3		1.217	0.040	0.066	1.650	0.426	0.075	0.112	1.493
V_{17}		1.289	0.022	0.033	1.500	0.444	0.050	0.064	1.280
O_1		-0.435	0.011	0.011	1.000	-0.156	0.015	0.019	1.267
O_2		-0.363	0.046	0.057	1.239	-0.133	0.039	0.049	1.256
O_5	BP/	-0.281	0.116	0.114	0.983	-0.144	0.114	0.106	0.930
O_6	DNP	-0.290	0.134	0.104	0.776	-0.164	0.127	0.093	0.732
O_{19}		-0.401	0.077	0.047	0.610	-0.162	0.076	0.043	0.566
O_{20}		-0.299	0.108	0.079	0.731	-0.160	0.101	0.067	0.663
V_3		1.216	0.040	0.065	1.625	0.421	0.075	0.111	1.480
V_{17}		1.289	0.022	0.034	1.545	0.441	0.050	0.066	1.320

7.4.5 Fukui function and relative electrophilicity:

We calculated the Fukui functions and relative electrophilicity values of selected atoms at the optimized geometry (Table 3). Although the condensed Fukui function values have been used to explain a variety of chemical properties[32], in the present context however, the f^+ and f^- failed to reproduce the experimentally observed intramolecular

reactivity trend. The failure of fukui functions (and/or local softness) to reproduce the experimental reactivity is not unprecedented[34,35].

Interestingly, the relative nucleophilicity and relative electrophilicity values are found to be very successful in predicting the experimental trend in many cases. The relative elecrophilicity values of peroxo oxygen atoms of the vanadium complex are given in Table 7.3. At PW91/DNP level of calculation the average relative electrophilicity value derived from Mulliken population scheme for the bridging peroxo group (O_1 and O_2, 1.074) is larger than that for the terminal peroxo groups (O_5, O_6, O_{19} and O_{20}, 0.771). The average f^+/f^- values calculated using Hirshfeld population scheme for the bridging and terminal peroxo groups are 1.215 and 0.719, respectively. In general the higher the f^+/f^- value of a group (atom) the higher is the susceptibility of the group (atom) to attack by a nucleophile (Br^- in the present context). The relative electrophilicity values calculated both by Mulliken Population and Hirshfeld population schemes clearly indicate higher reactivity of bridging peroxo group of the vanadium complex toward the Br^- ion which is in agreement with experimental observation. The BP/DNP calculated relative electrophilicity values also reproduce the experimental reactivity trend.

In summary, the results of the present study demonstrate that the bridging peroxo and side-on bound peroxo groups present in the complex investigated are nonequivalent structurally as well as in terms of their electrophilicity and hence reactivity. Formation of intramolecular H-bonding between the amino acid co-ligands imparts stability to the complex species. It is evident that greater electrophilicity of the O-atoms of VOOV moiety make them more susceptible to nucleophilic attack by the Br^- in an oxidative

bromination process, which presumably leads to the reductive cleavage of the O-O bond with concomitant oxidation of bromide. The observations are in harmony with the suggestion that a 'VOOV'group may be the principal requirement for bromide oxidation by peroxovanadate compounds, at neutral pH. However, in order to draw generalization of these views further theoretical investigations elaborating the electronic and energy characteristics of the reaction paths are required. Work in this direction is continuing in our laboratory.

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CHAPTER 8

Summary and Conclusions

Peroxovanadium chemistry, a thriving area of research is still on the upstream of the collective endeavor of contemporary interest due their potential catalytic[1-6], biochemical and therapeutic application[7-10] Within the context of the present work, there are certain aspects of the chemistry of peroxovanadate that appeared to have received relatively less attention. Information pertaining to polymer-anchored peroxovanadate seems to be very limited in spite of the importance and potential application of metal incorporated macromolecules in diverse areas ranging from catalysis to medicine[1-11]. Possible biochemical effect of pV bound macrocomplexes on enzyme function or their anti microbial activity are yet to be explored. Also, information on activity of discreet polymer anchored peroxovanadate in oxidative bromination is scanty[14]. In view of these observations, as part of the present research programme, we have endeavored to develop synthetic routes to stable and well defined peroxovandates anchored to soluble polymers and to derive information on some of their key properties of biological relevance, including their redox activity in biomimetic bromination. An important aspect has been to assess the relative relevance of the various factors, the most vital one being the nature of the ligand co-ordinated to the metal, which effect oxidant or biochemical activity of the synthesized peroxovanadium complexes[15,16]. An additional goal has been to undertake theoretical investigation on structure and reactivity of dinuclear peroxo bridged vanadium compound in order to understand the activity of 'VOOV' group in oxidative bromination.

In this Chapter, findings of our studies on peroxo chemistry of vanadium 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 new peroxo compounds of vanadium(V)

(i) Synthesis of well defined peroxovanadium compounds anchored to water soluble polymers possessing pendent functional groups such as carboxylate, maleate or sulphonate as potential ligand sites, can be achieved by reacting the respective polymer with V_2O_5 and H_2O_2 in aqous medium at near neutral pH. Maintenance of pH at ca.6 was found to be crucial for the desired synthesis.

(ii) The newly synthesized compounds $[V_2O_2(O_2)_4$(carboxylate)]-PA [PA = poly(acrylate)] (**PAV**), $[VO(O_2)_2$(carboxylate)]-PMA [PMA = poly(methacrylate)] (**PMAV**), PSS [PSS = Poly(sodium 4-styrene sulfonate)] (**PSSV**), $[V_2O_2(O_2)_4$(carboxylate)$VO(O_2)_2$(sulfonate)]-P(SS-*co*-M)[P(SS-*co*-M)= poly(sodium styrene sulfonate-*co*-maleate)] (**PSS-co-MV**) appear to be the first known exmples of water soluble peroxovanadates in macroligand environment. Also, the tripeptide containing pV $Na[VO(O_2)_2$(triglycine)$].3H_2O$ is a newer addition to the existing limited number of peroxovanadium compounds with biogenic co-ligands.

(iii) It is noteworthy that the carboxylate functional group present in the two closely related polymer matrices, poly(acrylate) and poly(methacrylate) binds the pV moieties in two different co-ordination modes leading to the formation of two predominant

structural forms of peroxovanadates viz., a dimeric tetraperoxovanadate in **PAV** and monomeric diperoxovanadate in **PMAV**.

(iv) The polymeric pV compounds viz., **PAV, PMAV, PSSV, PSS-co-MV** as well as monomeric complex **pV1** are stable towards decomposition in solutions of a wide range of pH values, including acidic pH, for a reasonable period of time. The presence of macro or peptide co-ligand in these complexes appears to enhance their stability probably through inter-ligand hydrogen bonding.

8.2 Biochemical properties of the polymeric and mononuclear pV compounds

(i) Interaction with catalase

This powerful enzyme leads to degradation of the compounds at rates almost 100 times slower compared to H_2O_2, the natural substrate of catalase. It is evident that synthesized polymeric pV complexes are relatively resistant to degradation under the effect of catalase action. The rates of degradation of the polymer bound compounds are also approximately 2-3 fold slower than that observed for monomeric hetero-ligand as well as free diperoxovanadate (DPV) under similar reaction condition.

(ii) Effect on alkaline phosphatase activity

- Each of the pV compounds tested irrespective of being monomeric or polymer-bound exert strong inhibitory effect on alkaline phosphatase activity with a potency significantly higher compared to that of the corresponding free ligand, vanadate and peroxovanadate. Effect of individual ligands on ALP activity is practically negligible under the assay conditions used and H_2O_2 as such had no observable effect.

- Kinetic studies revealed that the effect induced by the polymeric pV compounds on the enzyme is distinctly different from that observed for the monomeric complexes both in terms of potency as well as mode of inhibition. Each of the synthesized polymer bound peroxovanadates tested is a potent non-competitive inhibitor of rabbit intestine ALP, in contrast to the non-polymer bound DPV and heterolignad diperoxovanadates which showed mixed-type of inhibition of the enzyme. It is reasonable to expect the redox activity of the complexes to be one of the factors responsible for the type of inhibition exhibited by the compounds.

(iii) Effect of the pV compounds on bacterial growth

- Polymer-bound as well as free monomeric peroxovandium complexes tested, exhibit bactericidal effect against gram -negative *E. coli* and gram -positive *S. aureus*, with varying degrees of potency. Macro-complexes are observed to be more effective antibacterial agents in comparison to the monomeric pV compounds.
- Gram-positive *S. aureus* showed consistently greater sensitivity to the compounds relative to gram-negative *E. coli*. In addition to the difference in the cell wall characteristics of the two enzymes, nature of the ligand appears be one of the crucial factors due to which the two types of bacteria respond differently towards the compounds screened.
- The compound **PAV** with pV moieties bonded as dimeric species through bridging carboxylate groups of the polymer chain has been observed to be least potent as antibacterial agent, as well as ALP inhibitor among the four macro complexes examined.

The above results demonstrate that bioactivity of the pV compounds are sensitive not only to the nature of ancillary ligands, but also to the mode of co-ordianation of pV moieties to the pendant functional groups of the polymer chain. In absence of direct evidence at this stage however we are, unable to discern definitive reasons for the effect of the title compounds on the phosphatase activity or their antibacterial behaviour. Further investigation involving effect of other well-defined and stable peroxo-metallates in diverse ligand environment which remain intact in

solution, on the activity of the phosphatases and bacterial growth combined with speciation analysis are likely to help in establishing structure-activity correlation of the compounds and in gaining an insight into the mechanism involved in such enzyme inhibitory processes. This aspect is currently being studied in our laboratory.

8.3 Activity of the polymeric pV compounds in oxidative bromination

(i) The compounds **PAV** and **PSS-*co*-MV** with dimeric tetraperoxo vanadate moieties bonded to the polymer matrix, stoichiometrically oxidized bromide to a bromination competent intermediate in phosphate buffer at physiological pH. The bromination reaction can be made catalytic by the addition of exogenous H_2O_2.

(ii) These compounds are also efficient in mediating bromination of aromatic substrates in aqueous - organic media and afford regeneration.

(iii) Peroxovanadium compounds **PMAV** and **PSSV** are inactive in bromination. The findings are consistent with the proposal that for a pV complex to be active in bromination at neutral pH, a dimeric peroxovanadate configuration with a bridging peroxo group is the pre-requisite.

8.4 DFT studies on structure and reactivity of peroxobridged divanadate complex

(i) The findings of the theoretical investigation on dinuclear peroxobridged model compound[$V_2O_2(O_2)_3(glycine)_2$], shows that the bridging peroxo and side-on bound peroxo groups

present in the complex are nonequivalent structurally as well as in terms of their electrophilicity.

(ii) Results indicate the presence of strong intramolecular H-bond interaction in the complexes. The observation lends credibility to earlier suggestion that biogenic ancillary ligands stabilizes the highly reactive 'VOOV' species through inter-ligand interactions.

(iii) Greater electrophilicity of the O-atoms of VOOV moiety apparently make them more susceptible to nucleophilic attack by the Br^- in an oxidative bromination process.

8.5 Future prospects

The synthetic methodology developed to obtain the set of polymer bound peroxovanadate provides an efficient, straightforward pathway which opens opportunity to gain an easy access to similar water-soluble peroxo derivatives anchored to soluble polymers.

Taking into account the potent antimicrobial and enzyme inhibitory activity exhibited by the newly synthesized complexes we may expect the compounds to be of biochemical interest. The observation on the inhibitory effect of PMAV with respect to *S. aureus* may have important implications as this bacterium has been reported to be multi drug resistant. A distinctive feature of these pV compounds, which may be of clinical importance, is their reasonably high stability towards decomposition in solution of a wide range of pH values, particularly at acidic pH. This may be significant in view of the reports that orally administered

peroxovanadate was ineffective as anti diabetic drug in rats probably because it could not survive the strong acidity of the stomach. It is also notable that the degradation of the compounds under the effect of catalase is much slower compared to H_2O_2. Admittedly, the observed stability of these synthetic pV 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 suitable candidates for testing their properties for pharmacological applications.

One of the most notable aspects of the present study is the finding that the complexes **PAV** and **PSS-co-MV** besides being stoichiometric reagents for bromide oxidation in aqueous medium, also acted as catalyst for the same reaction when used in conjunction with H_2O_2 at neutral pH. The remarkable feature of the bromination protocol is that the reaction takes place under mild condition viz., at near neutral pH, in presence of relatively harmless bromide salt instead of bromine, and no extra addition of acid or H_2O_2 is required for the stoichiometric bromination reaction of the substrate. In addition, The compounds can be easily regenerated and reused. Thus it may be expected that information generated from the present investigation would be of interest in the context of developing bioinspired synthetic oxidants for organic bromination and hence would be relevant to Green Chemistry, a burgeoning area of current research[17,18].

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List of publications

Paper published in national and international Journals:

1. *Density functional study of structure and reactivity of a dinuclear peroxovanadate(V) complex*
   Diganta Kalita, Ramesh Ch. Deka and Nashreen S. Islam

   Inorg. Chem. Commun. 2007, 10, 45

2. *Synthesis, characterization, reactivity and antibacterial activity of new peroxovanadium(V) complexes anchored to soluble polymers*
   Diganta Kalita, Swapnalee Sarmah, Siva Prasad Das, Ashok Patowary, Diganta Baishya, Sashi Baruah, Nashreen S. Islam
   React. Funct. Polym., 2008, 68, 876

3. *Kinetics of inhibition of rabbit intestine alkaline phosphatase by heteroligand peroxo complexes of vanadium(V) and tungsten(VI)*
   Diganta Kalita, Siva Prasad Das Nashreen S. Islam
   Biol. Trace. Elem. Res.,

4. *Peroxovanadium complexes anchored to soluble polymers: synthesis, antibacterial behaviour and effect on alkaline phosphatase activity*
   Diganta Kalita, Siva Prasad Das, Jeena Jyoti Borooah, Alka Kumari, Sashi Baruah, Nashreen S. Islam (communicated)

5. *Synthesis of new dinuclear and mononuclear peroxovanadium(V) complexes containing biogenic co-ligands. a comparative study of some of their properties*
   Swapnalee Sarmah, Diganta Kalita, Pankaj Hazarika and Nashreen S Islam
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6. *Synthesis and characterization of novel catalase resistant monoperoxo divanadate(V) compounds*
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7. *New oxo-bridged peroxotungsten complexes containing biogenic co-ligand as potent inhibitors of alkaline phosphatase activity*
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8. *New oxo-bridged dinuclear peroxotungsten(VI) complexes. Synthesis, stability and activity in bromoperoxidation*
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Density functional studies on structure and reactivity of a dinuclear peroxovanadate(V) complex

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Abstract

Density functional method is used to investigate the structure and reactivity of peroxo-bridged divanadate complex $[V_2O_2(O_2)_3(glycine)_2]$ The results suggest the presence of two types of structurally non-equivalent peroxo groups and formation of hydrogen bonding between the glycine co-ligands in the complex These observations are in complete agreement with the experimental findings Density functional reactivity descriptors such as Fukui function and relative electrophilicity identify the bridged peroxo group of the complex as the most reactive one in terms of its susceptibility to attack by a neucleophile Attempt has been made to correlate the results of the study to the observed reactivity pattern of the complex species in oxidative bromination

Keywords Fukui function Relative electrophilicity Dinuclear peroxovanadate Oxidative bromination

Studies on synthetic peroxovanadate (pV) complexes as functional and structural models of bromoperoxidase (V-BPO) [1–7], the enzyme involved in the biosynthesis of a variety of naturally occurring brominated products, 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 [1–9] Contrary to natural V-BPO which is most efficient at pH 5 5–7, most of the synthetic peroxovanadate compounds studied as functional model of the V-BPO were found to be catalytically active only in acid medium [1,4,7] Significantly, a series of pV compounds with the distinctive feature of having a μ-peroxo group and amino acids or small peptides serving as ancillary ligands, of the type $[V_2O_2(O_2)_3(L)_3]\ H_2O$ (L = asn, gln, gly-gly, gly-ala or gly-asn) synthesized by us [10–12], could act as powerful bromide oxidant at physiological pH [10–13], an essential requirement of a biomimetic model Diperoxovanadate compounds with exclusively η^2-peroxo groups in its co-ordination sphere were found to be catalytically incompetent in bromide oxidation at neutral pH [12] The proposed reaction pathway based on our experiments and work of some other laboratories conferred the status of a selective bromide oxidant, at physiological pH, on VOOV group [10–15] The μ-peroxo group of the VOOV moiety appears to be amenable for reductive cleavage by bromide which produces a bromination competent intermediate, postulated to be $-BrOVO(O_2)$, that can transfer the bromine atom to the substrate [10–14] However, the cause of this greater reactivity of a peroxo-bridged divanadate moiety compared to a η^2-peroxo group, in an oxidative bromination reaction was not completely clear The crystal structures of these complexes could not be resolved so far due to their unstable nature in solution Reports related to synthetic dinuclear peroxovanadates with bridging peroxo group are very limited [10–12 16–19] probably due to the difficulties encountered in stabilizing such complexes

With an objective to gain an insight into the afore mentioned aspects, in the present work we investigated the structural and electronic properties of a dinuclear peroxo bridged vanadium complex $[V_2O_2(O_2)_3(glycine)_2]$ using

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density functional methods The initial structure of the complex has been modeled from the available spectroscopic and chemical analysis data related to the synthetic compound $[V_2O_2(O_2)_3(glycine)_2(H_2O)_2]$ [18] The structure has been optimized with PW91 and BP functionals without imposing symmetry constraints using the DMol^3 program [20] In fact we generated several initial structure of the peroxovanadium complex by changing the orientations of the ligands The structure shown in Fig 1 converged to a minimum with no negative vibrational frequency The calculated bond lengths and vibrational frequencies of O—O and V—O bonds have been compared with the available experimental and theoretical results We have also calculated the Fukui function and relative electrophilicity values using Mulliken and Hirshfeld population schemes in order to understand the reactivity of O—O moieties

A peroxo group bonded in a side-on fashion to V(V) center, exhibits strong ν(O—O) band at *ca* 870 cm^{-1} and ν_s and ν_{as}, which involve metal-oxygen stretches [21,22], appearing in the region 500–600 cm^{-1} (Table 1) In the spectra of the bridged peroxovanadate complexes in addition to the strong ν(O—O) absorption occurring at *ca* 835–870 cm^{-1}, a weak intensity but well resolved band has been observed at a lower frequency range of 805–820 cm^{-1} which has been assigned to the ν(O—O) band of the bridged peroxo group This observation was interpreted as an indication of the presence of two structurally different peroxo groups, the edge-bound and bridged type [10–12,18,19]

The PW91/DNP calculated vibrational frequencies are compared with experimentally determined frequencies in Table 1 The average error for frequencies calculated with B3LYP functionals was reported to be of the order of

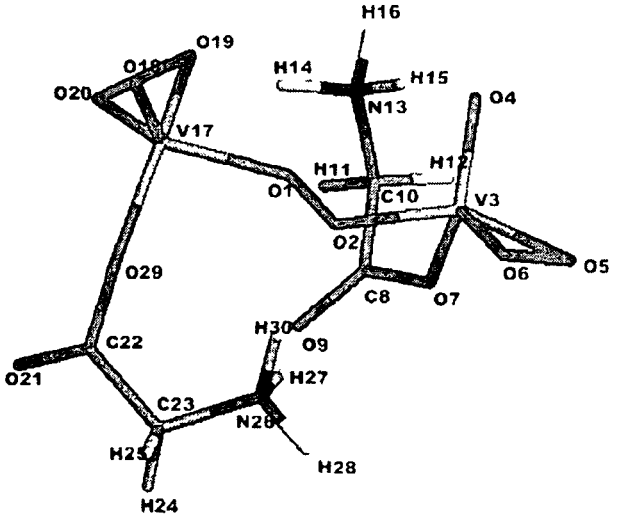

Fig 1 View of $[V_2O_2(O_2)_3(glycine)_2]$ complex showing six hydrogen bonds in the optimized geometry derived from PW91/DNP method The pairs which form hydrogen bonds are O_4—H_{15} O_9—H_{27} O_{19}—H_{14} O_2—H_{30} O_1—H_{14} and O_1—H_{30} Oxygen atoms O_1—O_2 form the bridge peroxide group whereas oxygen atoms O_5—O_6 and O_{19}—O_{20} form the two terminal peroxide groups

Table 1
Experimental and computed IR bands of the dinuclear peroxovanadate complexes indicating the presence of two kinds of O—O linkages

Complex	Infrared (IR) bands (cm^{-1})				Ref
	ν_s(V—O_2)	ν_{as}(V—O_2)	ν(O—O)	ν(V=O)	
$V_2O_2(O_2)_3$ (gly gly)$_3$ H_2O	561 m	613 m	835 s 803 w	958 s	[10]
$V_2O_2(O_2)_3$ (gly ala)$_3$ H_2O	572 m	620 m	835 m 803 w	949 s	[10]
$V_2O_2(O_2)_3$ (gly)$_3$ H_2O	562 m	631 m	859 s 803 m	954 s	[12]
$V_2O_2(O_2)_3$ (gln)$_3$ H_2O	562 m	640 m	870 m 809 m	955 s	[12]
$V_2O_2(O_2)_3$ (glyH)$_3$(H_2O)$_2$	530 m	605 m	835 m 820 w	959 s	[18]
	PW91/DNP Computed IR bands (cm^{-1})				
$V_2O_2(O_2)_3$(glyH)$_2$	566 m	635 m	923 s 834 w	983 s	

40–50 cm^{-1} for small inorganic molecules [23] In the present case therefore, in view of the relatively large size of the molecule, the discrepancy observed between calculated and measured frequencies appears to be acceptable Inspection of the data in Table 1 shows the presence of two kinds of O—O groups in the model complex viz, 923 and 834 cm^{-1} attributable to the edge-bound and bridging peroxo groups respectively

The PW91/DNP optimized geometry of the peroxo vanadate complex is presented in Fig 1 Oxygen atoms O_1—O_2 form the bridged peroxide group whereas oxygen atoms O_5—O_6 and O_{19}—O_{20} form the two terminal peroxide groups The selected geometrical parameters of the complex calculated at PW91/DNP and BP/DNP levels are presented in Table 2 It may be noted from Table 2 that the O_1—O_2 bond length is longer than that of the two terminal peroxide groups The average O—O bond lengths calculated at BP/DNP and PW91/DNP levels of calculations are 1 452 Å and 1 450 Å, respectively for the bridged and terminal peroxo groups which are in good agreement with the experimentally determined O—O bond length (1 424 to 1 503 Å) in pV complexes [1] The BP/DNP calculated average V—O bond length, 1 890 Å of V—O_2^{2-} group and average V=O bond length, 1 617 Å are also in conformity with the experimental values [1] of peroxo vanadium complexes (Table 2) Similar results are obtained at PW91/DNP level of calculation

Six hydrogen bonds involving amine hydrogens (H_{14}, H_{15} H_{30}), peroxo oxygens (O_1, O_2, O_{19}), carbonyl oxygen (O_9) and terminal oxo group (V=O_4) are observed in the optimized structure (Fig 1) The distances of all the six hydrogen bonds present in the complex are given in Table 2 The distances observed between the H_{14} (amine) and O_{19} (peroxo), H_{27} (amine) and O_9 (carbonyl), H_{15} (amine) and O_4 (terminal oxovanadium group V=O_4), and H_{30} (amine) and O_1 (peroxo-bridged O_2) of 1 597, 1 690, 1 794 and 1 827 Å, respectively, at PW91/DNP level of calculation indicate the presence of strong intramolecular interaction in the complex The presence of the H-bonds in the

Table 2
Selected geometric parameter of vanadium complex calculated at BP/DNP and PW91/DNP levels

Geometrical parameter[a]	Level of calculations		Available experimental values[b]
	BP/DNP	PW91/DNP	
O_1—O_2	1 458	1 455	
V_3—O_2	1 918	1 915	1.810–1 999
V_3=O_4	1 635	1 634	1 574–1 625
V_3—O_5	1.813	1.810	1 810–1 999
V_3—O_6	1 865	1 862	1 810–1 999
V_3—O_7	1 967	1 959	1 810–1 999
O_5—O_6	1 445	1 442	1 424–1 503
V_{17}—O_1	1 899	1 893	1 810–1 999
V_{17}—O_{18}	1.599	1 597	1 574–1 625
V_{17}—O_{19}	1.911	1 913	1 810–1 999
V_{17}—O_{20}	1.848	1 846	1 810–1 999
V_{17}—O_{29}	1 902	1 893	1 810–1 999
O_{19}—O_{20}	1 453	1 452	1 424–1 503
Hydrogen bonding			
O_4—H_{15}	1 811	1 794	
O_9—H_{27}	1 713	1 690	
O_{19}—H_{14}	1 619	1 597	
O_2—H_{30}	1 849	1.827	
O_1—H_{14}	2.391	2 384	
O_1—H_{30}	2 378	2 360	

[a] See Fig 1 for the atomic numbering Bond distances in Å
[b] From Ref [1]

compounds lend support to our earlier suggestion that the amino acid and peptide ligands impart stability to the highly reactive 'VOOV' species by inter-ligand interaction possibly hydrogen bonding, affording their isolation into solid state [10–13]. It is significant to note that such dimeric compounds could be isolated only in the presence of co-ligands with ability to form H-bond, such as amino acids and peptides. The dinuclear compounds although reasonably stable in the solid state undergo rapid degradation on dissolving in water with partial loss of peroxide [10–13], possibly due to the ready loss of hydrogen bonds in water

In order to understand why a μ-peroxo group of a dinuclear pV complex is so strongly activated toward oxidative bromination we performed theoretical calculations to derive atomic charges and DFT based reactivity descriptors Expecting that the net atomic charges of atoms in a molecule may provide valuable information about the reactivity of various atoms we derived the atomic charges of selected atoms of the complex using Hirshfeld and Mulliken schemes (Table 3) The oxygen atoms belonging to the terminal peroxo groups are observed to be the most negatively charged (−0.16, average value) followed by the bridged peroxo group (−0.14) as derived from Hirshfeld population scheme of PW91/DNP method. The BP/DNP calculated average Hirshfeld charges of bridged and terminal peroxo groups are also −0 14 and −0.16, respectively. The bridge peroxo group would therefore be expected to be the best candidate to interact with an electron rich site such as Br^-, according to the "electrophilic" mechanism proposed for the bromination reactions [1–4,13] mediated by vanadium complexes. However, the atomic charges derived from Mulliken population scheme did not exhibit a similar trend.

We calculated the Fukui functions and relative electrophilicity values of selected atoms of the optimized structure (Table 3) Although the condensed Fukui function values have been used to explain a variety of chemical properties [24], in the present context however, the f^+ and f^- failed to reproduce the experimentally observed intramolecular reactivity trend. The failure of fukui functions (and/or local softness) to reproduce the experimental reactivity is not unprecedented [25,26].

The relative electrophilicity values of peroxo oxygen atoms of the vanadium complex are summarized in Table 3 At PW91/DNP level of calculation the average relative electrophilicity value derived from Mulliken population

Table 3
PW91/DNP and BP/DNP calculated charges, Fukui functions and relative electrophilicity of selected atoms of the vanadium complex derived from Hirshfeld and Mulliken population schemes

Selected atoms		Mulliken population scheme				Hirshfeld population scheme			
		q	f	f^-	f^+/f	q	f	f^+	f^+/f
O_1	PW91/DNP	−0 440	0 011	0 010	0 909	−0 155	0 015	0 018	1 200
O_2		−0 362	0 046	0 057	1 239	−0 131	0 039	0 048	1 231
O_5		−0.278	0 114	0 114	1 000	−0 142	0 112	0 106	0 946
O_6		−0 289	0 132	0 103	0 780	−0 163	0 125	0 092	0 736
O_{19}		−0 402	0 079	0 047	0 595	−0 160	0 077	0.042	0.545
O_{20}		−0 300	0 110	0 078	0 709	−0 160	0 103	0 067	0 650
V_3		1 217	0 040	0 066	1 650	0 426	0 075	0 112	1 493
V_{17}		1 289	0 022	0 033	1 500	0 444	0 050	0 064	1 280
O_1	BP/DNP	−0 435	0 011	0 011	1 000	−0 156	0 015	0 019	1 267
O_2		−0 363	0 046	0 057	1 239	−0 133	0 039	0 049	1 256
O_5		−0 281	0 116	0 114	0 983	−0 144	0 114	0 106	0 930
O_6		−0 290	0 134	0 104	0 776	−0 164	0 127	0 093	0 732
O_{19}		−0 401	0 077	0 047	0 610	−0 162	0 076	0 043	0 566
O_{20}		−0 299	0 108	0 079	0 731	−0 160	0 101	0.067	0 663
V_3		1 216	0 040	0 065	1 625	0 421	0 075	0 111	1 480
V_{17}		1 289	0 022	0 034	1 545	0 441	0 050	0 066	1 320

scheme for the bridged peroxo group (O_1 and O_2, 1 074) has been observed to be larger than that for the terminal peroxo groups (O_5, O_6, O_{19} and O_{20}, 0 771) The average f^+/f^- values calculated using Hirshfeld population scheme for the bridging and terminal peroxo groups are 1 215 and 0 719, respectively In general the higher the f^-/f^- value of a group (atom) the higher is the susceptibility of the group (atom) to attack by a nucleophile (Br^- in the present context) The relative electrophilicity values calculated both by Mulliken population and Hirshfeld population schemes clearly indicate higher reactivity of bridged peroxo group of the vanadium complex toward the Br^- ion which is in agreement with experimental observation [10–13] The BP/DNP calculated relative electrophilicity values also reproduce the experimental reactivity trend

In summary, the results of the present study demonstrate that the bridging peroxo and side-on bound peroxo groups present in the complex investigated are nonequivalent structurally as well as in terms of their electrophilicity and hence reactivity Formation of intramolecular H-bonding between the amino acid co-ligands imparts stability to the complex species The greater electrophilicity of the O-atoms of VOOV moiety, evidently make them more susceptible to nucleophilic attack by the Br^- in an oxidative bromination process The observations are in harmony with the suggestion that a 'VOOV' group may be the principal requirement for bromide oxidation by peroxovanadate compounds, at neutral pH [10–14] However, in order to draw generalization of these views further theoretical investigation elaborating the electronic and energy characteristics of the reaction paths is required These aspects are planned to be presented in our future studies

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Synthesis, characterization, reactivity and antibacterial activity of new peroxovanadium(V) complexes anchored to soluble polymers

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Abstract

New peroxovanadate (pV) complexes anchored to soluble polymers of the type $Na_3[V_2O_2(O_2)_4$(carboxylate)]–PA [PA = poly(acrylate)] (PAV) and $Na_2[VO(O_2)_2$(carboxylate)]–PMA [PMA = Poly(methacrylate)] (PMAV) have been synthesized from the reaction of V_2O_5 with H_2O_2 and the sodium salts of the respective macromolecular ligands at pH *ca* 6 The compounds were characterized by elemental analysis SEM EDX, TGA and spectral studies In PMAV, the pV moieties are anchored in monomeric form to the polymer chain through unidentately co-ordinated *O* (carboxylate) atoms Carboxylate groups of PA chain co-ordinate to V(V) centres in a bridging bidentate fashion leading to the formation of dimeric pV structures in PAV The PAV complex efficiently mediated bromination of organic substrates in aqueous-organic media at ambient temperature Complex PMAV was inactive in bromination under analogous conditions The compounds are relatively resistant to degradation by the enzyme catalase compared to its natural substrate, H_2O_2 The polymeric complexes along with the free polymer and neat DPV were screened for their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*

Keywords Polymer bound peroxovanadate Dinuclear peroxovanadate Bromide oxidant Peroxidative bromination Antibacterial activity

1. Introduction

Peroxo complexes have been for the past several years object of intense investigation, for a variety of reasons including their role as oxidation catalyst [1,2], and biochemical relevance [1–5] Studies on synthetic peroxovanadate complexes as functional and structural models of bromoperoxidase, the enzyme involved in the biosynthesis of a variety of naturally occurring brominated products, have been immensely useful in elucidating the details of mechanism of action of the enzyme which is yet to be fully understood [1,6–12] Bromination of organic

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substrates, particularly aromatics, has been attracting considerable contemporary interest [13–21] mainly due to the commercial importance of such compounds Since the traditional bromination methods require the use of elemental bromine and solvents which are environmentally hazardous [22], there has been a continued search for alternative benign catalytic systems which can mimic the biological bromoperoxidase in the synthesis of brominated organics Contrary to natural V-BPO which is most efficient at pH 5 5–7 several model complexes were found to be catalytically active only in acid medium [1,6–10,23] which limits their utility as effective catalyst Some polymeric reagents are available as relatively safer stoichiometric halogenating agents, however their preparation require specific polymer backbone and direct contact with bromine [24,25]

Significantly, a series of pV compounds with the distinctive feature of having a μ-peroxo group of the type $[V_2O_2(O_2)_3(L)_3]\ H_2O$ (L = amino acid or dipeptide) synthesized by us [26,27] could act as powerful oxidant of bromide with good activity at physiological pH, thus mimicking the enzyme V-BPO Diperoxovanadate compounds with exclusively peroxo groups in its co-ordination sphere were found to be catalytically incompetent in bromide oxidation at neutral pH [26,28] The μ-peroxo V compounds however, undergo rapid degradation in solution with loss of its high bromination activity Nevertheless, the observation allowed to foresee potential of these compounds in oxidative bromination provided it can be prepared in a stable form

In the present work, we have therefore endeavored to design pV systems with enhanced stability and oxidative ability by anchoring active pV to polymer supports We were particularly interested to examine whether such polymeric compounds could act as oxidant of bromide with good activity at neutral pH, an essential requirement of a biomimetic model Activation and binding of dioxygen by polymer anchored transition metal is interesting from viewpoint of designing effective redox catalysts as well as modelling of complex processes in bio-systems [29,30] A few peroxometal systems [31–33] including one pV compound [34], supported on insoluble cross-linked polymers were prepared recently which were reported to exhibit good activity as catalytic or stoichiometric oxidant in organic oxidations [31–34] However, there appears to be no information available on peroxometallates bound to soluble polymers or use of such material as oxidative agent Despite the advantages of easy separation and regeneration of insoluble polymer supported reagent, such systems still exhibit several shortcomings due to the nature of heterogeneous reaction condition [35,36] The utility of soluble polymers as supports in organic chemistry is increasingly being recognized in recent years [35,36] One of the significant features of a soluble polymer supported reagent is the facility of product synthesis and characterization afforded by the soluble support due to the advantages of homogeneity offered it Accordingly, for our present study we have chosen soluble polymers viz , poly(acrylate) and poly(methacrylate) as supports since these are relatively cheaper, can be prepared conveniently or available commercially, and provide appropriate functional groups for easy attachment of active metal complexes Moreover, there has been considerable contemporary interest in development of pharmaceutical formulations consisting of poly(acrylic acid) and its derivatives [37–39] This particular aspect was important to us because an additional goal of the present investigation was to test the antimicrobial activity of the free and synthesized polymer bound pV compounds The antibacterial properties of hydrogen peroxide against Gram positive (G +ve) and Gram negative (G −ve) bacteria have been well established [40] Although potential usage of vanadium compounds as therapeutic anti-diabetic agents has been the subject of a number of recent reviews [3–5,41] and some vanadium compounds including decavanadate were reported to exhibit antibacterial activity [42–44], to the best of our knowledge, no peroxo compounds of vanadium has been tested for their antibacterial property

Reported in this paper are the first synthesis and characterization of polymer bound peroxovanadium compounds $Na_3[V_2O_2(O_2)_4(carboxylate)]$–PA [PA = poly(acrylate)] (PAV) and $Na_2[VO(O_2)_2$-(carboxylate)]–PMA [PMA = poly(methacrylate)] (PMAV) and studies on their stability and activity in oxidative bromination The results of antibacterial screening of the polymeric compounds against the G −ve *E coli* and G +ve *S aureus* are also reported herein

2. Materials and methods

2 1 Materials

The chemicals used were all reagent grade products The sources of chemicals are given below V_2O_5 (SRL, India), hydrogen peroxide (30%) (Ranb-

axy, New Delhi, India), phenol red (Merck, India Ltd), KBr, KH_2PO_4, K_2HPO_4 (SD Fine chemicals, India), Luria Agar, Luria Broth (HiMedia Laboratories, India) The sodium poly(acrylate) (M_w 2100) and catalase was obtained from Sigma–Aldrich chemical company Sodium polymethacrylate was prepared by solution polymerization technique Sodium diperoxovanadate (NaDPV) was prepared by the method described earlier [27] *E coli* MG1655 was grown and maintained in Luria Agar Culture was transferred 6–8 h prior to use to Luria Broth and incubated at 37 °C *S aureus* MTCC96 was grown and maintained in Nutrient Agar Culture were transferred 6–8 h prior to use to Nutrient Broth and incubated at 37 °C The water used for solution preparation was deionised and distilled

2 2 Synthesis of $Na_3[V_2O_2(O_2)_4(carboxylate)]$–PA (PAV) and $Na_2[(VO(O_2)_2(carboxylate)]$–PMA (PMAV)

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 36 g, 2 0 mmol) and 1 5 g of the respective polymer dissolved in minimum volume of water with continuous stirring Keeping the temperature below 4 °C in an ice bath, the mixture was stirred for *ca* 30 min until all solids dissolved The pH of the solution was recorded to be *ca* 3 Concentrated sodium hydroxide (*ca* 8 M) was added drop wise with constant stirring to raise the pH of the reaction medium to *ca* 6 On adding pre-cooled acetone (*ca* 50 ml) to this mixture under vigorous stirring a yellow colored pasty mass separated out After allowing it to stand for 20 min in the ice bath, the supernatant liquid was decanted and the residue was treated repeatedly with acetone under scratching until it became microcrystalline solid The product was separated by centrifugation, washed with cold acetone and dried in vacuo over concentrated sulfuric acid In the solid state these compounds were found to be stable for several weeks stored dry in closed containers at $\leqslant$30 °C

2 3 Elemental analysis

The compounds were analyzed for C and H at Regional Sophisticated Instruments Centre, North Hill University, Shillong, India Vanadium was estimated by the method mentioned in earlier papers [45] The total peroxide content was determined by adding a weighed amount of the compound to a cold solution of 1 5% boric acid (W/V) in 0 7 M sulfuric acid (100 ml) and titration with standard Cerium(IV) solution [45]

2 4 Physical and spectroscopic measurements

Spectra in the visible and ultraviolet region were recorded in a Cary 100 Bio, Varian spectrophotometer equipped with a peltier controlled constant temperature cell The absorbance values are denoted as, e g A_{592}, at the wave lengths indicated The infrared (IR) spectra were recorded with samples as KBr pellets in a Nicolet model Impact 410 and Perkin–Elmer model 983 FTIR spectrophotometer The spectra were recorded at ambient temperatures by making pressed pellets of the compounds The ^{1}H NMR spectra were recorded in deuterated chloroform in a Varian spectrophotometer using TMS as the internal standard for organic compounds Thermogravimetric analysis was done in Mettler Toledo Star system at a heating rate of 5 °C/min under an atmosphere of nitrogen using aluminum pan The SEM characterization was carried by using the LEO 1430 VP Scanning Electron Micrograph attached with energy dispersive X-ray detector Scanning was done at 10–20 μM range and images were taken at a magnification of 15–20 kV

2 5 Stability of the complexes in solution

Stability of the compounds in distilled water at pH *ca* 6 which is the natural pH of the compounds in solution, was studied by estimating the peroxide content in aliquots drawn from the solution of the compound PAV (0 11 mg/ml) or PMAV (0 14 mg/ml) compound at different interval of time by the method described under Section 2 3 As a measure of stability of the compounds in solution changes in absorbance of their electronic spectral band at *ca* 320 nm at ambient temperature were recorded at 30 min gap for a period of 24 h

2 6 Effect of catalase on the complexes

The effect of catalase on complexes was studied by estimating the peroxide content of the compounds in a solution containing catalase at specified time intervals The test solution contained phosphate buffer (50 mM, pH 7 0) and catalase (40 μg/ml) The volume of the reaction solution was kept at 25 ml The solution was incubated at 30 °C The

compound was then added to the test solution and aliquots of 5 ml were pipetted out and titrated for peroxide content after stopping the reaction by adding it to cold sulfuric acid (0 7 M, 100 ml) at time 5, 10, 30, 60 and 90 min of starting the reaction

2 7 Bromination of organic substrates

In a representative procedure, organic substrate (0 5 mmol) was added to a solution of acetonitrile water (1 1, 5 ml) containing Et_4NBr (2 mmol) A weighed amount of solid polymeric compound PAV (0 25 g) maintaining substrate pV at 1 1 was then added to the reaction mixture at room temperature under continuous stirring Progress of the reaction was monitored by TLC The stirring was continued for *ca* 7–10 h After completion of the reaction the 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 ^1H NMR spectroscopy and melting point determinations were made to interpret the products

2 8 Regeneration of the oxidant

The regeneration of the compound PAV for reuse was tested for the reaction using *p*-nitroaniline as substrate The reaction mixture contained the same recipe mentioned under Section 2 7 with a fourfold increase in the amounts of the components To regenerate PAV after completion of the reaction the following method has been adopted After extraction of the organic reaction product, the aqueous part of the reaction mixture was transferred to a 250 ml beaker Keeping the solution in an ice bath, 2 ml H_2O_2 was added to it maintaining the V peroxide at 1 4 followed by addition of excess DMF with constant stirring until a yellow colored mass separated out After allowing it to stand for 5 min in the ice bath, the supernatant liquid was decanted off and the residue was treated repeatedly with DMF and distilled acetone until it became yellow microcrystalline solid The product was separated by centrifugation, washed with cold acetone and dried in vacuo over concentrated sulfuric acid

2 9 Measurement of bromination activity in solution

The method of de Boer et al [46] of introducing four bromine atoms into the molecule of phenol red ($\varepsilon^{433} = 19\,7$ mM) to form a bromophenol blue ($\varepsilon^{592} = 67\,4$ mM) was used to measure bromination activity The reaction mixture contained phosphate buffer (50 mM, pH 5 5), KBr (1 M) and phenol red (20 μM) The redox activity was tested by adding a measured amount of aliquot from complex solution and by monitoring the possible change in the absorbance at 592 nm at 30 °C The volume of the reaction mixture was kept at 25 ml Aliquots were transferred to the spectrophotometer immediately after mixing

2 10 Assessment of antibacterial activity

The antibacterial activity against *Staphylococcus aureus* (*S aureus*, G +ve) and *Escherichia coli* (*E coli*, G −ve) were determined by the method of plate counting Typically 1 ml of bacterial suspension containing 2–5 × 10^6 cells/ ml were incubated with either medium alone (positive control) or predetermined concentration of test compound in a final volume of 1 ml at 37 °C for 45 min Thereafter the cells were diluted and plated onto agar plates and CFU counted after overnight incubation at 37 °C The antibacterial effect was estimated by calculating% inhibition using the formula

$$\% \text{ Inhibition} = C - T/C \times 100$$

where C is the mean of CFU in control and T is the mean of CFU in presence of given concentration of test compounds The number of CFU was calculated by multiplying the numbers of colonies with the dilution factor

The MIC values were determined as the minimum concentration of the inhibitor compound at which complete inhibition of the bacterial growth was observed in comparison to the control, disregarding single colony or a faint haze caused by the inoculums

3. Results and discussion

3 1 Synthesis

One of the advantages of using a soluble polymeric ligand for the synthesis of polymer-bound metal complexes is the possibility of adopting synthetic procedures used for preparing their low molecular weight analogues The methodology for the successful synthesis of the polymer anchored peroxovanadates PAV and PMAV was based on the reaction of V_2O_5 with H_2O_2 and Na salt of the respective polymeric ligand in an aqueous medium

Chelation of a metal ion by a polymeric ligand such as polycarboxylate, as well as composition of peroxo-vanadium species have been known to be sensitive to pH of the reaction medium [47–50] In the present study, the strategically maintained pH of *ca* 6 was found to be conducive for the desired synthesis of the compounds The alkali used to raise the pH of the reaction solution also served as source of counter cation for the complex anions The procedure included other essential components such as maintenance of required time and temperature at ≤4 °C and limiting water to that contributed by 30% H_2O_2 and alkali hydroxide solution Solvent precipitation is a valuable and general way to isolate soluble polymer bound compounds [35,36] The compounds were isolated by addition of acetone which facilitated the precipitation

3 2 Characterization

The elemental analysis data of the compounds PAV and PMAV (Table 1) indicated the presence of two peroxide groups per metal centre The V loading on the compounds based on elemental analysis and confirmed by EDX spectral analysis was found to be 2 07 mmol for PAV and 1 59 mmol for PMAV, respectively per gram of polymeric support

3 2 1 SEM and energy dispersive X-ray (EDX) analysis

Scanning electron microscopy was used to investigate the morphological changes occurring on the surfaces of the polymers after loading of the peroxovanadates to the polymer chains In contrast to the smooth and flat surfaces of the pure poly(acrylate) or poly(methacrylate) polymers, the surfaces of the polymer anchored complexes exhibited considerable roughening (Fig 1) Energy dispersive X-ray spectroscopy of the compounds, which provides in situ chemical analysis of the bulk, clearly showed V, C, O and Na as the constituents of the anchored complexes (Fig 2) Moreover, the EDX spectral data obtained on the composition of the compounds were in good agreement with the elemental analysis values

3 2 2 IR and electronic spectral studies

The IR spectra of the polymer bound peroxo complexes PAV and PMAV displayed a sufficiently well resolved spectral pattern significant features of which are summarized in Table 2 and presented in Fig 3 The presence of side-on bound peroxo ligand in the compounds, was evident from the observance of the characteristic ν(O–O), ν_{asym}(V–O_2) and ν_{sym}(V–O_2) modes, in the vicinity of *ca* 870, *ca* 610 and *ca* 530 cm^{-1}, respectively [51] The spectra enabled clear identification of ν(V=O) near 960 cm^{-1} arising from terminally bonded V=O group [51 52] The spectral pattern attributable to peroxovanadate moiety compared very well with the one observed for free DPV

The IR spectra showed characteristic differences between the spectral pattern originating from polymer-metal complexes and spectra of the free polymer Previous investigations on the interaction of poly(acrylate) and metal ions reveal that the frequency difference between the symmetric and antisymmetric stretches ($\Delta\nu = \nu_{asym} - \nu_{sym}$) of the carboxylate group in the polymer coordinated complexes compared to the free polymer can be made use of to determine the mode of attachment between the carboxylate group and the metal centre [53,54] In free sodium poly(acrylate) (NaPA) ν_{asym}(COO) and ν_{sym}(COO) modes are observed at 1565 and 1409 cm^{-1}, respectively ($\Delta\nu = 156\ cm^{-1}$). In the spectra of PAV, a slight shifting of the ν_{asym}(COO) band to a higher frequency of 1573 cm^{-1} was observed however, the $\Delta\nu$ remained close to that observed for free NaPA The observation clearly

Table 1
Analytical data of the polymer-bound peroxovanadate complexes

Compound	% Found from elemental analysis (% obtained from EDX spectra)					Metal ion loading [a] (mmol g^{-1} of polymer)
	C	H	Na	V	O_2^{2-}	
1 $Na_3[V_2O_2(O_2)_4$(carboxylate)]-PA	22 31	6 29	–	10 57	13 2	2 07
	(23 49)	–	(13 48)	(9 51)	–	
2 $Na_2[VO(O_2)_2$(carboxylate)]-PMA	28 77	7 20	–	8 13	10 11	1 59
	(29 12)	–	(11 4)	(7 9)	–	

[a] Metal ion loading = $\frac{\text{Observed metal \%} \times 10}{\text{Atomic weight of metal}}$

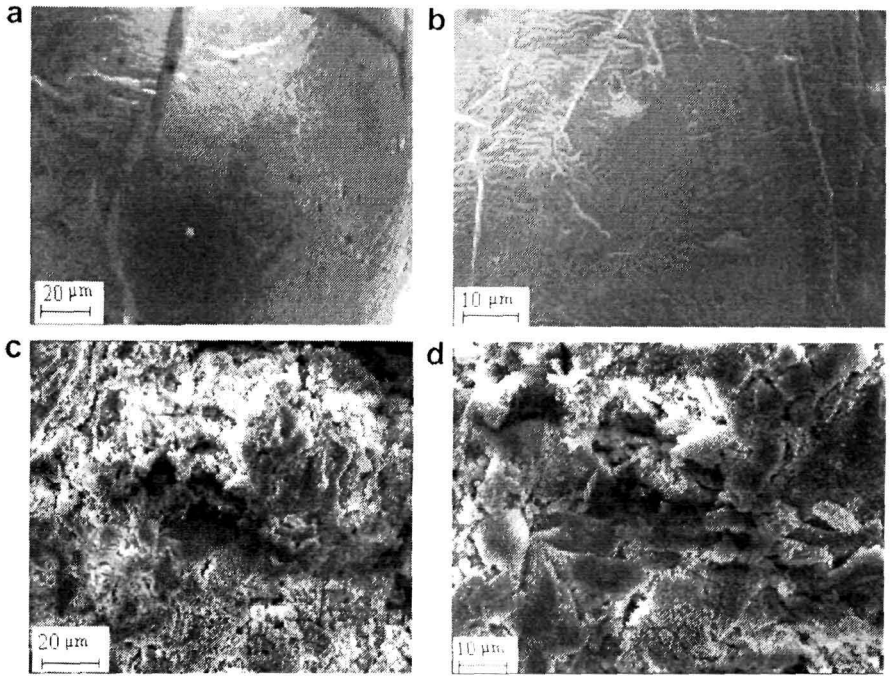


Fig. 1. Scanning electron micrographs of (a) sodium poly(acrylate), (b) sodium poly(methacrylate), (c) PAV and (d) PMAV.

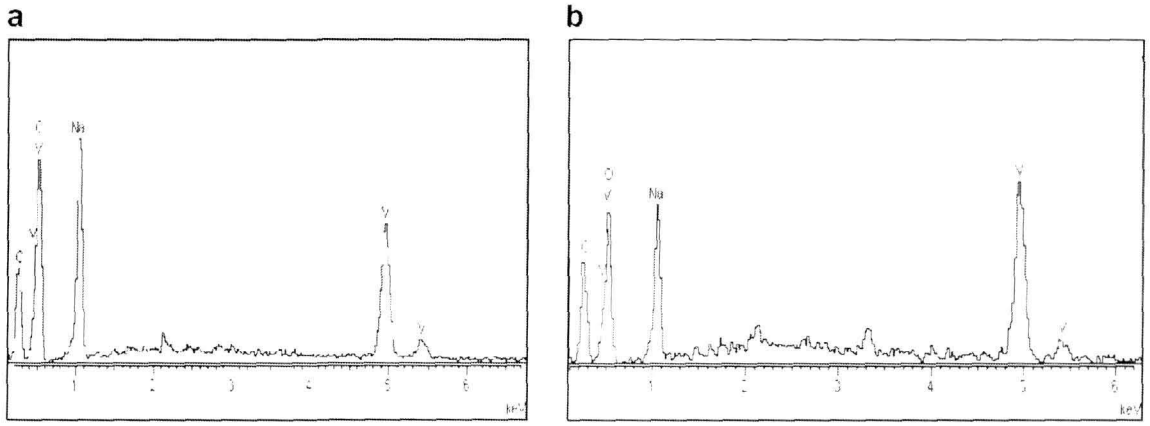


Fig. 2. EDX spectra of (a) PAV and (b) PMAV.

Table 2
Infrared spectral data of the peroxovanadate complexes PAV and PMAV

Compound	IR peaks (cm^{-1})				UV peak (nm)
	ν(V–O_2)	ν(V O_2)	ν(O–O)	ν(V=O)	
1. $Na_3[V_2O_2(O_2)_4$(carboxylate)]–PA	525	618	872	969	322
2. $Na_2[VO(O_2)_2$(carboxylate)]–PMA	526	637	875	966	320
3. DPV	522	602	877	935	325

suggested that the poly(acrylate) chain through its carboxylate group co-ordinate to V(V) in a bidentate bridging fashion. In case of the PMA bound peroxovanadate PMAV, the bands attributable to ν_{asym}(COO) and ν_{sym}(COO) appeared at 1660 and 1406 cm^{-1}, respectively. The distinct shift of the

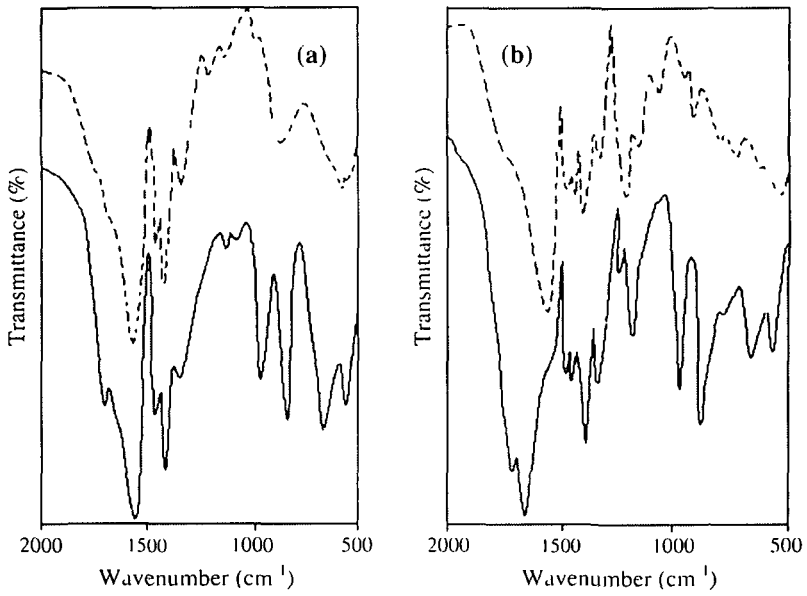


Fig 3 IR spectra of (a) PAV (solid line) and NaPA (broken line) and (b) PMAV (solid line) and NaPMA (broken line)

ν_{asym}(COO) to a higher frequency and that of ν_{sym}(-COO) to a lower frequency compared to the corresponding free polymeric ligand values of 1540 and 1415 cm^{-1} is typical of monodentate co-ordination of the carboxylate group Weak bands in the far IR region between 500 and 400 cm^{-1} have been assigned to metal oxygen vibrations The IR spectral data thus provided clear evidence for the bonding of V-peroxo moiety to PA and PMA in two different fashions In each of the compounds PAV and PMAV the presence of free -COOH groups was evident from the additional band appearing at *ca* 1712 cm^{-1} region The spectra of the compounds exhibited the characteristic bending $-CH_2$ mode at *ca* 1465 cm^{-1} Occurrence of lattice water in each of the complexes was apparent from the appearance of strong ν(OH) absorptions displayed at 3500–3400 cm^{-1}

Depending on the pH of the reaction solution and the nature of the metal a carboxylate group can act as a monodentate, bidentate chelating or bidentate bridging ligand [55] It is quite intriguing to note that the carboxylate functional group present in the two closely related polymer matrices, poly(acrylate) and poly(methacrylate) binds the pV moieties in two different co-ordination modes leading to the formation of two structural forms of peroxovanadates viz , a dimeric tetraperoxovanadate in PAV and monomeric diperoxovanadate in PMAV This difference in co-ordination pattern may be attributed to the presence of $-CH_3$ groups attached to the polymer backbone in PMA which being relatively bulkier probably prevents the formation of a dinuclear pV species through carboxylate bridge.

The electronic spectra of the compounds PAV and PMAV in aqueous solution displayed a weak intensity broad band at 320 nm which was assigned to peroxo to vanadium (LMCT) transition The band was observed in the range characteristic of a diperoxovanadate(V) species [26]

On the basis of the above results the proposed structure of polymer anchored pV complex in PAV, that includes two V atoms co-ordinated to the polymer chain through a bidentate bridged carboxylate group, side-on bound peroxo ligands and terminal V=O, is shown schematically in Fig 4a For PMAV, a structure of the complex species, incorporating unidentate co-ordination of carboxylate of the polymer to a diperoxovanadate unit has been envisaged (Fig 4b)

3 3 Thermal analysis

Thermogravimetric analysis data indicated that after the initial dehydration, the compounds undergo multistage decomposition (Table 3) The TGA curve of PAV is presented in Fig 5 The thermograms of the two compounds PAV and PMAV showed the first stage of decomposition occurring between 40 and 90 °C with the liberation of mole-

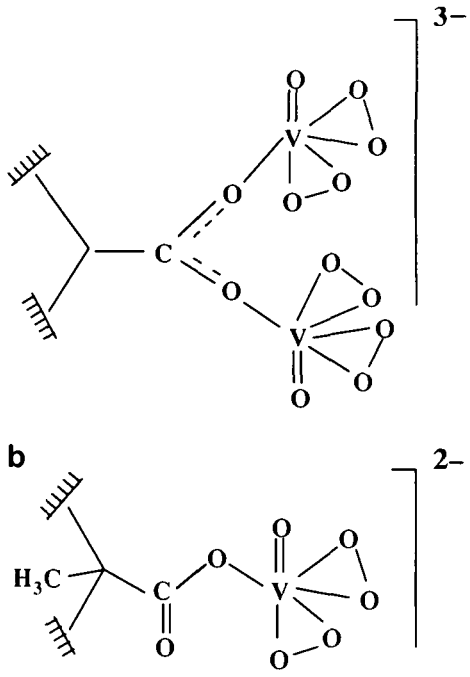


Fig 4 Proposed structures of (a) $Na_3[V_2O_2(O_2)_4$(carboxylate)]–PA (PAV) and (b) $Na_2[VO(O_2)_2$(carboxylate)]–PMA (PMAV) "▬" represents polymeric support

cules of water of crystallization from the complexes with a corresponding weight loss of 12.5% (PAV) and 16.0% (PMAV). The next decomposition stage is in the temperature region of 90–250 °C for PAV and 90–180 °C for PMAV with a corresponding weight loss of 13.0% and 10.0%, respectively attributable to complete loss co-ordinated peroxo groups from the complexes. Absence of peroxide in the decomposition product, isolated at this stage, was confirmed from the IR spectral analysis. The loss of peroxide is seen to be followed by a three stage decomposition occurring in the broad temperature range of 290–800 °C for PAV and 310–600 °C for PMAV respectively which may be attributed to decarboxylation involving free as well as coordinated carboxylate functionals accompanied by rupturing of the polymers [56,57]. Further evidence regarding decarboxylation of the polymers was obtained from the IR spectra recorded after heating the compounds separately up to a temperature of 600 °C which showed complete disappearance of the strong peaks originating from ν_{asym}(COO) and ν_{sym}(COO) modes in the 1600 cm^{-1} to 1400 cm^{-1} region of the spectra of the title compounds. The total weight loss which occurred during the course

Table 3
Thermogravimetric data of complexes

Compound	Temperature range (°C)	Observed weight loss (%)	Final residue (%)
1 $Na_3[V_2O_2(O_2)_4$(carboxylate)]–PA	40–90	12.5	
	90–250	13.0	
	290–800	38.5	36.0
2 $Na_2[VO(O_2)_2$(carboxylate)]–PMA	40–90	16.0	
	90–180	10.0	
	310–600	30.0	44.0

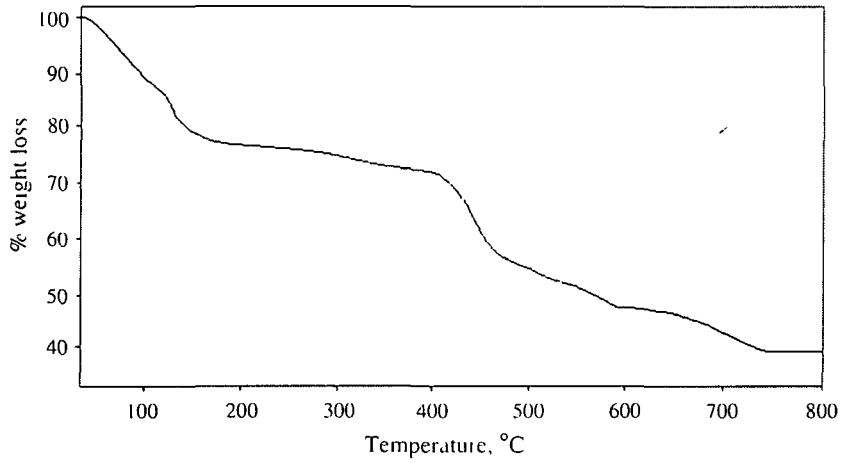


Fig 5 TG plot of PAV

of the overall decomposition process on heating the compounds up to a final temperature 800 °C was recorded to be 64 0% for PAV and 56 0% for PMAV due to the complete loss of the components viz , lattice water, coordinated peroxide and polymeric functionals The IR spectra of the residue remaining at this stage showed the presence of oxovanadium species Thermogravimetric analysis data of the compounds thus provided further evidence in support of the composition and formula assigned to the compounds

3 4 Stability of the PAV and PMAV in solution – their action with catalase

The investigations on the stability of the compounds in an aqueous solution of pH *ca* 6, which is the natural pH attained by the solution of the polymer bound compounds PAV and PMAV in water, revealed that their peroxide content and position and intensity of their electronic spectral bands remained unaltered for over a period of 24 h We further examined and ascertained their stability in solutions of pH values ranging from 3 6 to 8 0 (data not shown) Fig 6 shows that the compound PAV used as a representative, is stable in solution of pH 6 0 as well as at pH 7 0

Keeping in view our goal of studying some of the biochemically relevant properties of the complexes, we considered it imperative to examine the sensitivity of the newly synthesized pV complexes towards

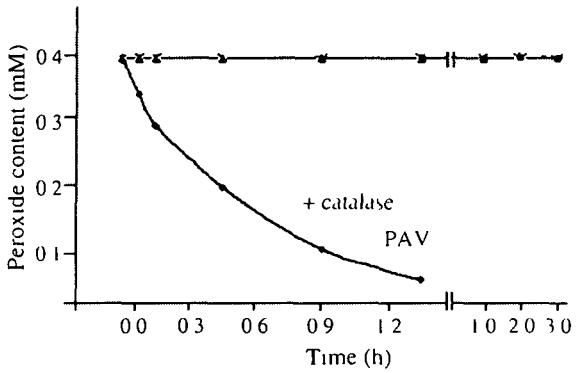


Fig 6 Stability of compound PAV at different pH values effect of catalase × Compound solution in distilled water (0 11 mg/ml) pH of the solution 6 0 ▲ Solution of complexes in phosphate buffer (pH 7 0) ♦ Effect of catalase The test solution contained phosphate buffer (50 mM pH 7 0) and the catalase (40 μg /ml) which was incubated at 30 °C for 5 min Compounds (0 11 mg/ml) were then added to the reaction solution and aliquots were drawn at indicated time points and loss in peroxide content was determined

catalase, the ubiquitous enzyme that catalyze the breakdown of H_2O_2 formed during oxidative processes in the intercellular peroxisomes On incubation with catalase, each of the compounds PAV and PMAV was found to be degraded slowly with the loss of peroxide The effect of catalase on the complex PAV is shown in Fig 6 Total peroxide loss from each of the compound solutions tested having equivalent concentrations of co-ordinated peroxide (0 4 mM) was recorded to be *ca* 0 4 mM, indicating a ratio of 1 4 for peroxide dinuclear pV species (PAV) and 1 2 for mononuclear pV (PMAV) which are in excellent agreement with the estimated peroxide content of the compounds

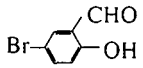
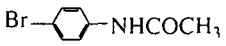
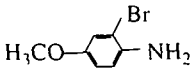
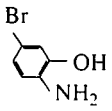
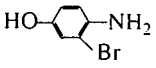
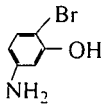
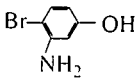
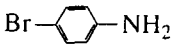
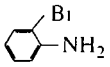
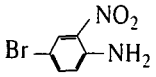
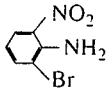
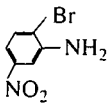
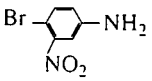
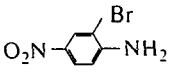
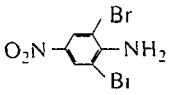
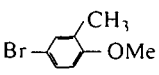
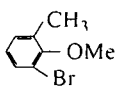
The rates of degradation of the polymer bound diperoxovanadates PAV and PMAV under the effect of catalase action were found to be 5 8 and 5 6 μM/min, respectively which are approximately half of that observed for free diperoxovanadate (DPV) (12 0 μM/min from a solution of 0 2 mM) under similar reaction condition [58] Under the effect of catalase the rate of degradation of H_2O_2 with the release of oxygen was reported to be 430 μM/min from a solution of 0 1 mM concentration and the reaction will be completed in less than 2 min It is thus evident that the synthesized polymer anchored pV complexes are at least 50 times weaker as substrates to catalase compared to H_2O_2, its natural substrate The relatively greater resistance of the compounds to the powerful enzyme catalase may be attributed to the additional stability imparted to the compounds by the polymeric support

3 5 Redox activity of the compounds in oxidative bromination

3 5 1 Substrate bromination in aqueous-organic media

Brominations of several activated aromatics into their corresponding bromo-organics were achieved simply by stirring a solution of the substrate in presence of PAV in CH_3CN H_2O (1 1) for 7–10 h at ambient temperature (Table 4) Tetraethyl ammonium bromide (Et_4NBr) was used as source of bromide The condition of reactions such as reaction temperature substrate oxidant stoichiometry, bromide concentration and type of solvent were optimized using the substrate *p*-nitroaniline as a representative A 1 1 oxidant substrate stoichiometry appeared to be optimal and the solvent CH_3CN H_2O (1 1) provided good yields of the

Table 4
Bromination of organic substrates mediated by compound PAV

Substrate	Product		% Yield	Time (h)
Salicylaldehyde			80	10
Acetanilide			82	10
Anisidine			75	8
o-Aminophenol			83	8
p-Aminophenol			85	8
m-Aminophenol			a 77, b 18	8
Aniline			a 80 b 15	8
o-Nitroaniline			a 70, b 23	7
m-Nitroaniline			a 65, b 30	7
p-Nitroaniline			a 75, b 21	7
o-Methoxytoluene			a 60 b 25	8

products Activated aromatics such as aniline and acetanilide were brominated to produce predominantly *p*-bromo products The remarkable feature of the present methodology is that the reaction takes place at near neutral pH and no extra addition of acid or H_2O_2 is required for the stoichiometric bromination reaction of the substrate It was intriguing to note that under the optimized conditions the neat DPV complex was totally inactive in bromination and PMAV showed poor oxidant activity affording the brominated products in <5% yield

Preferential bromination at either *ortho* or *para* position of the aromatic ring leading to mono substitution indicates an electrophilic bromination mechanism That the brominating species was 'Br^{+}' and not a Br radical in these reactions was further evident from the ring substituted products obtained from the substrate, 2-methoxytoluene (Table 4) Bromination through radical reaction would have produced benzyl bromide instead of bromo-methoxy toluene

3 5 2 *Regeneration of the reagent*

Most frequently, recovery of the soluble polymer supported reagent can be achieved by diluting the homogeneous polymer solution by the addition of an excess of a poor solvent which induces precipitation of polymeric species [35,36] The resulting heterogeneous mixture is filtered to obtain the polymer For satisfactory recovery of a soluble polymer from reaction mixture proper choice of solvent and temperature are crucial

In the present study, regeneration of the oxidant PAV was accomplished easily by treating the aqueous extract of the spent reaction mixture with H_2O_2, maintaining the peroxide V ratio at 1 4, followed by the addition of DMF to this solution at ambient temperature which induced complete precipitation of the polymer-bound complex Since Et_4NBr used in excess as a bromide source in the reaction is soluble in DMF, possibility of co-precipitation of this species along with the polymer could be ruled out Addition of hydrogen peroxide was necessary in order to compensate the peroxide consumed during the bromination reaction The IR spectrum of the recovered product was identical with the original starting complex which showed the presence of side-on bound peroxo group and bridged carboxylate group Reuse of the recovered complex in a fresh cycle of bromination reaction afforded the desired brominated products indicating that metal complexes are intact with the polymer chain and the oxidant is active even after the first cycle However, a slight decrease in product yield obtained suggested the possibility of leaching of the metal over subsequent reuse of the compound

3 5 3 *Activity of the complexes in bromination in aqueous solution*

With an objective to gain a better understanding of the reactivity of the compound PAV in mediating oxidative bromination, we considered it imperative to further investigate the reaction in solution in line with our earlier work on bromination activity of peroxovanadates [26,27,59]. The bromination of phenol red to its tetra brominated product, bromophenol blue [46] was used to measure the bromination activity of the complex PAV in solution Addition of freshly prepared aqueous solution of PAV (0 11 mg/ml) to the standard reaction of bromide in phosphate buffer with phenol red as trap for oxidized bromine resulted in gradual color change of the solution from yellow to blue The spectrum recorded showed a peak at A_{592} characteristic of the product bromophenol blue and a decrease in absorbance of the peak at A_{433} due to loss of phenol red (Fig 7) The initial rate of bromine transfer under the assay conditions used as obtained from the plot was 3 1 μM Br/min The constant rate of the reaction during which A_{592} progressively increased was preceded by an initial lag It was of interest to note that the bromination activity shown by PMAV was practically negligible and DPV as such was totally inactive in bromination (Fig 7)

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 solution of compound PAV Addition of phenol red to this solution resulted in the decrease in A_{262} nm and a peak at 592 nm appeared indicating the formation of bromophenol blue The 262 nm peak, therefore, represents a bromination competent oxidized species of bromide, probably an equilibrium mixture of BrOH, Br_2 and Br_3^- as proposed earlier [46]

While the initial addition of H_2O_2 (0.5 mM) to the reaction solution had no observable effect on the initial rate of bromination, a revival of the bromination activity was noted on addition of H_2O_2 (0 5 mM) to the spent reaction mixture which contained excess bromide and substrate. The reaction thus could be made catalytic by the addition of exogenous hydrogen peroxide which is apparently required for in situ regeneration of the active brominating species

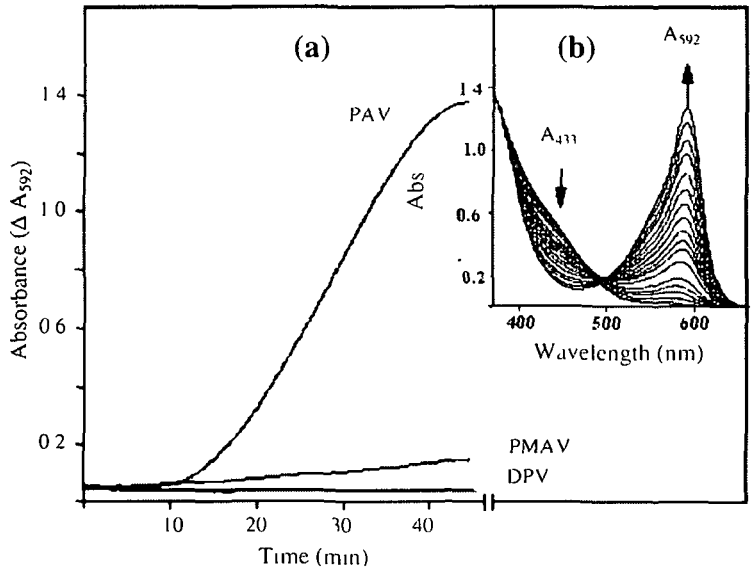


Fig 7 Bromination activity with PAV (a) The increase of A_{592} for bromophenol blue indicating the rate of bromination of phenol red with PAV The reaction mixture contained phosphate buffer (0 05 M pH 5 5) KBr (1 M) and phenol red (20 μM) PAV (0 11 mg/ml) (b) Spectral changes at 2 min interval following bromination of phenol red to bromophenol blue on addition of compound solution to the reaction mixture are shown in the inset

By itself, H_2O_2 is capable of oxidizing bromide in highly acidic medium (pH <3) but is ineffective in solution at pH >5 0 The reaction is catalyzed by vanadium compounds such as $VOSO_4$ [60] and V_2O_5 [11] However, synthetic DPV or monoperoxovanadate or compounds containing such moieties were catalytically incompetent in bromide oxidation at neutral pH [61] The observation that inactive DPV requires the presence of vanadyl or vanadate to gain oxidant activity at pH >5, led to the proposal that μ-peroxo-bridged divanadate intermediate, $[OVOOV(O_2)]^{2+}$, formed by complexation between these two species is the proximate oxidant of bromide [28] Support for such an intermediate came from our investigations on a series of synthetic compounds with a VOOV bridge which were found to be active in oxidative bromination at neutral pH [26,27,62] Based on these findings it was suggested earlier that a μ-peroxovanadate group may be the principal requirement for bromide oxidation by pV compounds at neutral pH [26.27,62]

The above observations are relevant in explaining the observed activity of the polymer bound dimeric tetraperoxovanadate compound PAV in oxidative bromination and inactivity of PMAV in the same reaction It is reasonable to expect that anchoring of the pV in the form of a dimer to the polymeric chain where two pV units are held together in close proximity by a bridging carboxylate, would facilitate the formation of a peroxobridged species which then can act as bromide oxidant The initial lag in the reaction may be interpreted in terms of the time required for the formation of the peroxo-bridged intermediate in solution In PMAV compound with relatively lower V loading and pV units distributed over the polymer matrix as monomeric DPV, such bridge formation is unlikely which probably explains its inactivity in bromination Thus the findings of the present study are in accord with the earlier suggestion that for a pV complex to be active in bromination at neutral pH a μ-peroxovanadate configuration is the pre-requisite

3 6 *Antibacterial activity*

The effect of PAV and PMAV compounds on growth of bacteria viz , G −ve *E coli* and G +ve *S aureus* was examined by the method of viable counting from which percent inhibition was calculated and compared with the effect induced separately by the free polymeric supports and free DPV A significant decrease in number of colony forming units (CFU) of *E coli* ($p < 0\ 05$) as well as of *S aureus* ($p < 0\ 005$) was noted at concentrations ⩾100 μg/ml of PAV (Fig 8) The minimal inhibitory concentration (MIC) of PAV calculated from the data for *E coli*

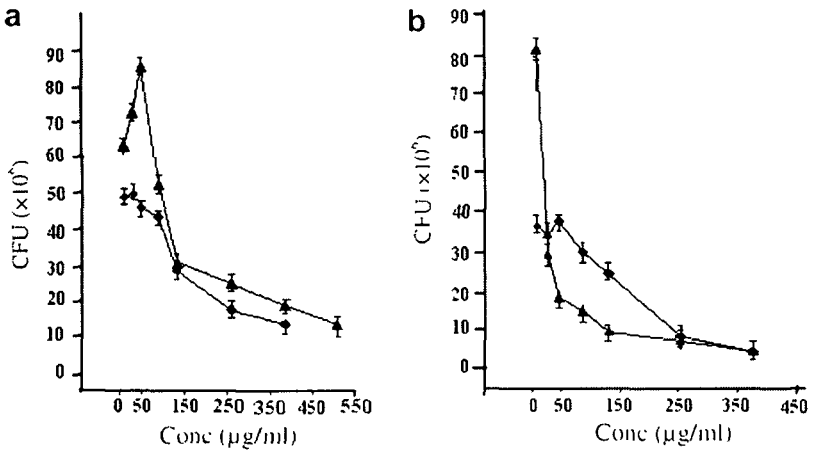


Fig 8 Antibacterial activity of the compounds PAV (♦) and PMAV (▲) against (a) *E coli*, (b) *S aureus* The data were the means ± SE of three parallel experiments that were done in triplicate

and of *S aureus* was 375 μg/ml and 500 μg/ml. Significantly, PMAV treatment resulted in 66.8% inhibition of *S aureus* at concentration of 20 μg/ml but equivalent inhibition of *E. coli* was seen at concentrations greater than 125 μg/ml. Although, the two bacteria responded differently to lower concentrations of PMAV, the MIC for both bacteria was noted to be 375 μg/ml. Further, this antibacterial activity against *E. coli* was specific. It is notable that under our experimental conditions used the effect of free polymers on bacterial growth was practically negligible Free DPV showed antibacterial activity for both *E coli* and *S aureus* (MIC 129 μg/ml). On comparing these values with MIC of polymer bound complexes in terms of their actual pV content at these concentrations (Table 5) it was apparent that DPV was considerably less active than PAV and PMAV. It is therefore evident that among the tested species the polymer bound pV compounds are the most active antibacterial agents. It may thus be inferred that anchoring of pV species to the polymer enhances the antibacterial properties of the compounds.

Table 5
Antibacterial activity of the complexes against *E coli* and *S aureus*

Compound	MIC (μg/ml) (containing μg equivalent of DPV)[a]	
	S aureus	*E coli*
1 PAV	500 0	375 0
	(119 0)	(89 2)
2 PMAV	375 0	375.0
	(68 5)	(68 5)
3 DPV	129 0	129 0

[a] Calculated on the basis of pV loading

One of the remarkable observation was that PMAV showed more than 50% inhibition of *S aureus* at concentration as low as 20 μg/ml (containing 3 67 μg equivalent of pV), whereas nearly ten times higher concentration of 200 μg/ml of PAV was found to result in comparable inhibition of the bacterium. The higher sensitivity of *S aureus* to PMAV is surprising as this compound has markedly lower percent of the active peroxovanadate moieties compared to PAV The difference in antibacterial property of the two polymeric compounds may be attributed to the structural differences of the compounds which are likely to influence their bioavailability, as well as the difference in the outer envelope characteristics of the two bacteria [63]. It may be expected that the ability of the two compounds to penetrate through or interact with bacterial cell wall would differ due to factors such as presence of $-CH_3$ group in the PMA chain, difference in ionic charge distribution and the polarity of the two compounds as a consequence of coordination of the pV species in PMAV and PAV in two different modes. It is tempting to suggest that the relatively lower charge on PMAV would facilitate better interaction of this species with G +ve cell wall of *S aureus* resulting in to cell membrane damage and cell death as is the case with antibacterial defensins which are cationic peptides. The greater inhibitory effect of the anchored pV compounds in comparison to free DPV is probably because of slower degradation of polymer bound pV as evident from their activity with catalase. More investigations are required to explain the findings and in absence of direct evidence at this stage we refrain

from drawing any conclusion regarding definitive cause of the antibacterial effect of the title compounds Nevertheless the results suggest the possibility of modulating the bioactivity of a pV compound by modifying the coordination environment, providing with a scope to design antibacterial agents more active against a group of bacteria

4. Conclusions

In conclusion, pV compounds anchored to soluble polymers poly(acrylate) and poly(methacrylate) have been isolated and tested for their activity in organic bromination The newly synthesized PAV complex could serve as stoichiometric reagent for bromide oxidation in aqueous-organic medium at neutral pH, whereas PMAV was inactive in bromination In view of the easy method of preparation of the reagent, redundancy of bromine or hydrobromic acid in the bromination process mediated by it, and environmentally acceptable reaction condition, we may expect PAV to emerge as a useful addition to the range of biomimetic synthetic oxidants in bromide oxidation The result of the investigation is consistent with the proposal implicating formation of a peroxo-bridged divanadate intermediate which is active in bromination An additional distinctive feature of the compounds is their high stability in solution of a wide range of pH values and their relative resistance to degradation by the powerful enzyme catalase Another significant finding is the antibacterial effect exhibited by free DPV as well as polymer bound PAV and PMAV The observation on the inhibitory effect of PMAV with respect to *S aureus* may have important implications as this bacterium has been reported to be multi drug resistant Our results demonstrate that the activity of these pV compounds are sensitive to the co-ligand and that even minor modification of the co-ordination environment can alter the properties of the complexes tremendously

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