Synthesis and Studies on Peroxotungsten(VI) Compounds Bound to Macromolecules. Towards Development of Bio-Relevant Complexes and Active Oxidation Catalysts

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By

SIVA PRASAD DAS

Registration No. 002 of 2009

Department of Chemical Sciences
School of Science and Technology
Tezpur University
Tezpur ,Assam

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Dedicated to -

My Parents

ABSTRACT

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ABSTRACT

The present thesis deals with the results of studies involving synthesis, characterization and stability of some new peroxotungsten (pW) complexes anchored to water soluble, as well as insoluble polymer supports. The thesis also provides an account of the findings of investigation on the interaction of the complexes with certain enzymes and their activity as catalyst or stoichiometric oxidant in mild organic oxidations. The contents of the thesis have been compiled into seven chapters.

Chapter 1 presents a general introduction pertaining to the work embodied in the thesis. The importance of and the interest in tungsten chemistry and biochemistry in general, and peroxotungsten(VI) compounds in particular are highlighted. Apart from the importance of insoluble polymers, the utility of water soluble, biocompatible polymers as supports in chemistry and biology is being emphasized. Attention is being drawn to the paucity of information on polymer supported peroxometallates and to the fact that potential of discreet pW compounds as biologically active agents remains relatively unexplored despite the knowledge that tungstate display favourable bio-relevant characteristics.

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 activities of the complexes in oxidative bromination, sulfide oxidation and interactions with various enzymes are described herein.

In **Chapter 3,** synthesis of a series of diperoxotungsten(VI) complexes bound to water soluble polymers of the type, $[WO(O_2)_2(carboxylate)]$-PA [PA = poly(sodium acrylate)] (**PAW**) (**3.2**), $[WO(O_2)_2(carboxylate)]$-PMA [PMA = poly(sodium methacrylate)] (**PMAW**) (**3.3**), $[WO(O_2)_2(amide)]$-PAm [PAm = Poly(acrylamide)] (**PAmW**) (**3.4**) and $[WO(O_2)_2(sulfonate)]$–PS [PS = poly(sodium vinylsulfonate)] (**PSW**) (**3.5**) are described. The compounds were generated under reasonably mild condition from the reaction of H_2WO_4, respective macromolecular ligand and 30% H_2O_2 at pH *ca.* 5. An insoluble polymer immobilized monoperoxo compound, $[WO_2(O_2)(CN)_2]$-PAN [PAN = poly(acrylonitrile)] (**PANW**) (**3.1**) was obtained by employing poly(acrylonitrile) as support and slight variation of the reaction conditions. Synthesis of the compounds, in addition to pH, is sensitive to reaction temperature and concentrations of the components.

The compounds were characterized by elemental analysis (CHN and energy dispersive X-ray spectroscopy), spectral studies (UV-Vis, IR and ^{13}C NMR), thermal (TGA) as well as scanning electron micrographs (SEM) analysis. In **PANW (3.1),** the pW moieties are anchored to the polymer matrix through the N atom of the pendant nitrile group. The compounds **PAW (3.2)** and **PMAW (3.3),** contain pW moieties unidentately co-ordinated via O (carboxylate) atoms. In the compounds **PAmW (3.4)** and **PSW (3.5)** the pW species are similarly attached to the macromolecular ligand through N (amide) or O (sulfonate) atoms, respectively in unidentate fashion. It is notable that in **PANW (3.1),** the pW groups are attached in its dioxomonoperoxo form in contrast to the soluble complexes, where they occur as oxodiperoxotungsten(VI) species.

The stability of the compounds in solution of a wide range of pH values ranging from 1.2 to 8.0 has been assessed. It has been demonstrated that the compounds retain their structural integrity in solution of acidic as well as higher pH.

Chapter 4 deals with the results of investigations on the reactivity of the polymer bound peroxotungstate complexes, $[WO_2(O_2)(CN)_2]$-PAN **(3.1)**, $[WO(O_2)_2$(carboxylate)]-PA **(3.2)**, $[WO(O_2)_2$(carboxylate)]-PMA **(3.3)** and $[WO(O_2)_2$(amide)]-PAm **(3.4)** in oxidation of organic sulfides. Attempt has been made to document the comparison between the activity of the two classes of the compounds investigated viz., insoluble polymer-immobilized, **PANW (3.1)** and water soluble polymer-anchored analogues, **PAW (3.2), PMAW (3.3)** and **PAmW (3.4)**.

Selective oxidation of a variety of sulfides and dibenzothiophene (DBT) to the corresponding sulfoxide or sulfone, using H_2O_2 as oxidant, could be achieved at room temperature in presence of the heterogeneous as well as homogeneous polymer-anchored catalyst, **PANW (3.1)**, **PAW (3.2), PMAW (3.3)** and **PAmW (3.4)**, by a versatile variation of reaction conditions. The reactions proceed under mild conditions to afford the resulting products with impressive turnover frequency (TOF). However, the heterogeneous catalyst **PANW (3.1)** displayed superior activity compared to the homogeneous analogues. Redundancy of chlorinated solvent or any other additive, are the additional important attributes of the protocol.

Each of the peroxotungsten(VI) complexes examined exhibit complete chemoselectivity toward sulfur group of substituted sulfides with other oxidation prone functional groups. Moreover, all the polymer-anchored catalyst afforded easy regeneration and can be reused with consistent activity and selectivity for at least up to seven reaction cycles. However, the catalytic activity of the homogeneous polymer-

anchored catalysts, **PAW (3.2), PMAW (3.3)** and **PAmW (3.4)** was observed to decrease with increasing number of cycles. Redundancy of chlorinated solvent or any other additive, are the additional important attributes of the protocol.

Presented in **Chapter 5**, are the findings of our investigation on activity of mononuclear as well as dinuclear diperoxo complexes of tungsten of the type, $[WO(O_2)_2$(glycyl-glycine)$].3H_2O$ (**MWG**), $Na_2[W_2O_3(O_2)_4$(glycyl-glycine)$_2].3H_2O$ (**DWG**) or $Na_2[W_2O_3(O_2)_4$(cystine)$].4H_2O$ (**DWC**) as selective oxidant for sulfoxidation reaction. An efficient method for the selective oxidation of a variety of structurally diverse sulfides to sulfoxides, under environmentally clean conditions, has been developed using monomeric diperoxotungsten complex, **MWG (5.1)** as well as anionic dinuclear tetraperoxo tungsten complexes, **DWG (5.2)** and **DWC (5.3)** as stoichiometric oxidants. The compounds could also efficiently catalyze the selective oxidation of sulfides by H_2O_2 to yield sulfone with reasonably good TOF, under mild reaction conditions. The simplicity in the method of preparation of the reagents and excellent chemoselectivity displayed by the reagents towards sulfur group of substituted sulfides are important advantageous features of the methodology. Compounds can be stoichiometrically recovered in the presence of H_2O_2.

Reported in **Chapter 6** are the reactivity of the supported peroxotungstate complexes, $[WO_2(O_2)(CN)_2]$-PAN **(3.1)**, $[WO(O_2)_2$(carboxylate)]-PA **(3.2)**, $[WO(O_2)_2$(carboxylate)]-PMA **(3.3)** and $[WO(O_2)_2$(amide)]-PAm **(3.4)** in oxidative bromination. Bromination of organic substrates, particularly aromatics, has been attracting considerable contemporary interest from both biological and chemical

perspectives. There has been a continued search for alternative benign catalytic systems which can mimic the biological bromoperoxidase in the synthesis of brominated organics.

Bromination of several activated aromatics was achieved simply by stirring a solution of the substrate in presence of the soluble compounds **PAW (3.2), PMAW (3.3), PAmW (3.4)** and **PSW (3.5)** in CH_3CN:H_2O (1:1), at ambient temperature and by using KBr or Et_4NBr as bromide source instead of elemental bromine. The insoluble monoperoxotungsten(VI) compound was ineffective as stoichiometric oxidant, however, it efficiently catalysed the oxidative bromination of organic substrate by H_2O_2 in reasonably good TOF. Each of tested compounds afforded regeneration and could be reused in fresh cycles of bromination.

Bromination activity of the complexes in aqueous solution was examined by employing phenol red as substrate. The complexes **PAW (3.2), PMAW (3.3), PAmW (3.4)** and **PSW (3.5)** effectively oxidized bromide to a bromination competent intermediate in phosphate buffer at physiological pH, an essential requirement of a biomimetic model. Addition of freshly prepared compound solution to standard reaction of bromide resulted in gradual colour change of the solution from yellow to blue. The spectrum recorded displayed 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. A revival of the bromination activity of the water soluble polymer-anchored compounds 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. Based on the results, a mechanistic pathway implicating the formation of an inactive monoperoxo tungsten intermediate has been formulated.

Chapter 7 deals with the results of investigations on the activities of the soluble polymeric complexes $[WO(O_2)_2(carboxylate)]$-PA (**3.2**), $[WO(O_2)_2(carboxylate)]$-PMA (**3.3**), $[WO(O_2)_2(amide)]$-PAm (**3.4**) and $[WO(O_2)_2(sulfonate)]$–PS (**3.5**) with the enzymes, catalase and phosphatases. The effect of two previously reported monomeric heteroligand peroxotungsten(VI) complexes of the type, $[WO(O_2)_2(glycyl\text{-}glycine)(H_2O)].3H_2O$ (**5.1**) and a dimeric compound $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ (**5.3**) on the activity of acid phosphatase has been reported herein. Comparisons between the two sets of peroxotungsten compounds viz., monomeric free complexes and polymeric ones could be drawn with respect to their tested properties. To the best of our knowledge, this is the first report where discreet pW compounds are examined for their inhibitory effect on acid phophatases.

The effect of the above mentioned peroxotungsten complexes upon two different membrane bound phosphatases viz., wheat thylakoid membrane acid phosphatase (ACP) and rabbit intestine alkaline phosphatase (ALP), were tested by employing established enzyme assay system and p-NPP (p-nitrophenylphosphate) as substrate. Each of the tested complexes behaved as active inhibitors of the function of the model enzymes. The two classes of enzymes however, exhibited significantly different sensitivity towards the inhibitors. The IC_{50} and K_i values were more than 50 orders of magnitude lower for ACP than those observed for ALP showing a greater affinity of the complexes for the enzyme binding site of ACP compared to ALP. The kinetic data enabled us to group the complexes into two classes on the basis of their distinct mechanistic preferences. The group comprising of polymeric pW complexes behave as classical non-competitive inhibitors for ACP as well as ALP while the free heteroligand pW compounds show mixed-type of inhibition combining competitive and non-competitive pathways. The results show that there is a marked influence of the co-ligand environment on the inhibitory potency of the intact metal complexes.

The effect of catalase, the reactive oxygen (ROS) mopping enzyme responsible for breakdown of H_2O_2 in the intercellular peroxisomes, on complexes was studied vis-a-vis its natural substrate H_2O_2, 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 polymer-anchored 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 polymer-anchored peroxotungsten complexes are several fold weaker substrates to catalase as compared to H_2O_2, its natural substrate.

The major part of the results of studies described in **Chapters 3-7** have been published and the rest is under communication.

Declaration

I hereby declare that the thesis entitled "***Synthesis and Studies on Peroxotungsten(VI) Compounds Bound to Macromolecules. Towards Development of Bio-relevant Complexes and Active Oxidation Catalysts***" 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.

Siva Prasad Das /

Place: Tezpur University (Siva Prasad Das)

Date: 31/3/12.

TEZPUR UNIVERSITY

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NAPAAM, TEZPUR-784028

DISTRICT :: SONITPUR :: ASSAM :: INDIA

Ph: 03712-267004
03712-267005
Fax: 03712-267006
03712-267005

I certify that the thesis entitled "***Synthesis and Studies on Peroxotungsten(VI) Compounds Bound to Macromolecules. Towards Development of Bio-relevant Complexes and Active Oxidation Catalysts***" submitted to the Tezpur University in the Department of Chemical Sciences under the School of Science and Technology, in partial fulfillment for the award of the degree of Doctor of Philosophy in Science is a record of research work carried out by Mr. Siva Prasad Das under my supervision and guidance.

All help received by him from various sources have been duly acknowledged.

No part of this thesis has been submitted elsewhere for award of any other degree.

Place: Tezpur University
Date: 31.3.12

N. S. Islam
(Prof. Nashreen S. Islam)
Professor
Department of Chemical Sciences
School of Science and Technology

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Department of Chemical Sciences
Tezpur University
Date: 31/3/12

Siva Prasad Das

(Siva Prasad Das)

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List of Abbreviations

ACP	acid phosphatase
ALP	alkaline phosphatase
AH	acetylene hydratase
AOR	aldehyde oxidoreductase
DBT	dibenzothiophene
dmf	dimethyl formamide
DNA	deoxyribonucleic acid
DTG	differential thermogravimetry
EDTA	ethylenediaminetetraacetic acid
EDX	energy dispersive X-Ray analysis
FDH	Formaldehyde:ferredoxin-oxidoreductase
GC	gas chromatography
HIV	human immunodeficiency virus
HMPA	hexamethylphosphoric triamide
hmpt	hexamethylphosphorous triamide
HPLC	high Performance Liquid Chromatography
gly-gly	glycyl-glycine
IC_{50}	half-maximal inhibitory concentration
IR	infra red
insRTK	insulin receptor tyrosine kinase
LMCT	ligand to metal charge transfer
MPS	methyl phenyl sulfide
MPV	monoperoxovanadate
ndt	2,3- naphthalenedithiolate
NMR	nuclear magnetic resonance
ODS	oxidative desulfurization
PA	poly(sodium acrylate)
PAm	poly(acryl amide)
PAN	poly(acrylonitrile)
PEG	poly(ethylene glycol)
PEI	polyethyleneimine

PMA	sodium polymethacrylate
PMMA	poly(methylmethacrylate)
PSNa	poly(sodium vinyl sulfonate)
pic	picolinato
p-NPP	p-nitrophenyl phosphate
p-NP	p-nitrophenol
PTC	phase transfer catalyst
pV	peroxovanadate
P4VP	poly(4-vinyl pyridine)
pW	peroxotungstate
PTPase	phosphotyrosine phosphatase
RT	room temperature
RNA	ribonucleic acid
SEM	Scanning electron microscopy
TG	thermogravimetry
TLC	thin layer chromatography
TMB	1,3,5-trimethoxybenzene
TOF	turnover frequency
V-BPO	vanadium bromoperoxidase
V-HPO	vanadium haloperoxidase

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CHAPTER 1

Introduction

1.1 TUNGSTEN – HISTORICAL PERSPECTIVE AND OCCURRENCE

Tungsten, also known as Wolfram, belongs to Group VI of the periodic table having ground state electron configuration [Xe] $4f^{14}\ 5d^4\ 6s^{2p\ 1\text{-}3}$. The name originates from the Swedish words "tung sten" meaning "heavy stone" and its symbol W from its former name Wolfram. In 1758, Axel Fredrik Cronstedt, a Swedish chemist and mineralogist, discovered and described an unusually heavy mineral which he called "tung-sten"[4,5]. He was convinced that this mineral contained a new and, as yet undiscovered, element[4,5]. It was not until 1781 that a fellow Swede, Carl Wilhelm Scheele, succeeded in isolating the oxide (tungsten trioxide)[1,4,6]. In 1783, two Spanish chemists, the brothers Elhuyar de Suvisa, first reduced the mineral wolframite to tungsten metal. Jöns Jacob Berzelius, in 1816 and later, in 1824, Friedrich Wöhler described the oxides and bronzes of tungsten and gave the name "wolfram"[1,6]. The metallurgy of tungsten was started in 1847 when R. Oxland took out a patent for the manufacture of sodium tungstate and tungstic acid. Thus Oxland was marked as the real founder of the tungsten chemistry[4,6].

Tungsten metal is silvery-white and lustrous, but the element is usually obtained as a grey powder. It is ranked 54^{th} in natural abundance[7]. The average abundance of tungsten on earth is approximately 1ppm (μg/g)[1]. In ocean water, its abundance is 2.0×10^{-5} mg/litre [8]. Tungsten occurs in the natural state only in the form of chemical compounds with other elements. Although more than twenty tungsten bearing minerals

are known, only two of them are important for industrial use, namely wolframite (Fe, Mn)WO_4 and scheelite $CaWO_4$. Presence of tungsten in some biological microorganism has also been established with the recent discovery of several tungstoenzymes[9-13].

Tungsten and its alloys are used extensively for making filaments for electrical lamps and electron tubes[1]. The element has the highest melting point (3380 °C) and lowest vapor pressure of all metals, and at temperatures over 1650 K has the highest tensile strength owing to which W is used in various high temperature applications[1-3].

1.2 BIOLOGICAL SIGNIFICANCE OF TUNGSTEN

Tungsten is the bioelement with the highest atomic number, 74, and the only bioelement in the third transition row of the periodic table. Although tungsten is widely distributed in biology, however, it is not a universal bioelement. Tungsten in many ways is the twin element of molybdenum. The atomic and ionic radii and the chemical properties of tungsten are very similar to those of molybdenum. They can catalyze reactions such as hydroxylation of carbon centres under more moderate conditions than are required by other systems[14]. Also in biology the coordination chemistries of Mo and W are similar in structural and functional aspects. It is therefore surprising that while the essential role of molybdenum in biology has been known for decades and molybdoenzymes are ubiquitous[10-15] yet, the evidence for involvement of W in biological systems could be obtained only recently. Tungsten was till recently treated only as a molybdenum antagonist, since replacement of Mo by W leads to inactivation of Mo containing enzymes[15]. However, the development of biochemistry of W has now become an endeavour in its own right.

The first major breakthrough in this area came in the year 1983 with the report on isolation and purification of the first naturally occurring tungstoenzyme from one of the acetogens although, it has been known since 1970s that tungstate stimulated the growth of certain acetate and methane-producing microorganisms[16-18]. During the last decade the partial characterization of a number of oxygen-sensitive, pterin-containing tungsten enzymes from thermophiles has stimulated a renewed interest in the bioinorganic chemistry of tungsten[19,20]. At present, more than a dozen tungstoenzymes have been purified[13,21] and the crystal structure of one of them has been determined[22]. The physiological roles of these enzymes are fundamental, and include the catalysis of key steps in carbon, nitrogen and sulfur metabolism[23,24]. Tungstoenzymes are grouped into three categories[13,22,25]. Members of the AOR and F(M)DH families catalyze redox reactions, whereas, AH type catalyze hydration of acetylene. A few tungsten based model complexes for the three types of enzyme viz. AOR, FDH and AH has been prepared[10]. However, none of these reported complexes can clearly demonstrate the reactivity of the enzyme functions[10]. Summarized in Table 1.1 are the types of tungstoenzymes and the reactions catalyzed by them[13,26].

Table 1.1 Representative examples of the reactions catalyzed by Tungstoenzymes[13,26]

Enzyme	Reactions catalyzed	Metal and cofactor content, mol/mol protein	Ref.
Formate dehydrogenase	$HCO_2^- \rightleftharpoons CO_2 + H^+ + 2e^-$	W (2) Se (2) Fe/S (20-40)	27
Formaldehyde:ferredoxin -oxidoreductase	CO_2 activation	W (4) FeS (4)	28,29
Aldehyde: oxidoreductase	$RCHO + H_2O \rightleftharpoons RCO_2H + 2H^+ + 2e^-$	W (2) FeS (4)	25
Acetylene hydratase	$HC \equiv CH + H_2O \rightleftharpoons CH_3CHO$	W (0.5) Fe (3) S (4)	30

A fascinating finding which enhanced the awareness of pharmacological value of tungsten and its compounds is the report of Goto et al. in a 1992 on the ability of tungstates[31], like vanadates to mimic the biological action of insulin[32].The known insulin mimetic effects of tungstate[33] led to polyoxotungstate clusters being evaluated as insulin mimetics in animal models[34]. Foster and co-workers reported that tungstate acts as one of the most potent competitive inhibitor of multifunctional glucose-6-phosphatase hydrolysis known[35,36]. Moreover, enzyme inihibitory effect of tungstates on protein phosphatases activity was documented by them[36]. Another exciting development was initiated with the observation of Claret et al. that tungstate prevent body weight gain in high fat diet-induced obese rates leading to reduced adiposity and improved insulin sensitivity[37]. These results focused tungstate as a promising new therapeutic agent for the treatment of obesity[37]. In addition, compounds of tungsten such as polyoxotungstates, particularly silicotungstates have been recently reported to show antiviral activity[35,38,39] and were found to be potent inhibitors of HIV reverse transcriptase and RNA-dependant DNA polymerase[39]. Despite these important findings and the fact that toxicity of W is relatively low, there appears to be very few studies devoted to the biochemical studies or exploration of therapeutic potential of compounds of tungsten[32,35,40,41]. Concomitant with renewed biological interest there has been an increasing interest in elucidating the chemistry of tungsten complexes as its co-ordination chemistry play a central role in the interaction with biomolecules[32,42-44] as well as in catalytic oxidations[45-51].

1.3 CO-ORDINATION CHEMISTRY OF TUNGSTEN – SELECTED ASPECTS

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.

The chemistry of tungsten coordination compounds is exceptionally complicated[1]. The reasons are: (i) Tungsten form complexes in oxidation states ranging between -2 to +6. (ii) The coordination number is variable which can go up to maximum of 13. (iii) Tungsten has the tendency to form clusters and polynuclear complexes[1] with varying number of atoms. In these compounds the tungsten-to-tungsten bond varies between single and quadruple. The lower the valence state the higher the degree of W-W bonding. The dilute aqueous speciation of tungsten in water is probably completely dominated by the tungsten(VI) oxoanion WO_4^{2-} over a wide range of pH and redox potential values[9,52] that would cover conditions found in most of the terrestrial inhabitable environments. At increased concentrations a kinetically, and thermodynamically complex polyoxoanion chemistry evolves[9,53]. The biologically significant oxidation states of tungsten are +4, +5 and +6 [1,13,26].

Hexavalent tungsten can be found in the form of hexahalides, which give rise to a variety of substitution products containing the structural unit W^{6+}. In addition, hexavalent tungsten shows a strong tendency to form bonds of higher order than one with donor atoms like O, S, Se or N. Consequently, distinction has to be made between complexes of the structural units W^{6+}, WO^{4+} and WO_2^{2+} and the analogous complexes which contain =S, =Se or =NR instead of oxygen[1]. The possible donor atoms and the corresponding ligands encountered in tungsten compounds are presented in Table 1.2. Thiotungstate ions

like WS_4^{2-}, WOS_3^{2-} and $WO_2S_2^{2-}$ act as bidentate ligands for other metal ions. A prominent feature of the chemistry of tungsten is the formation of polytungstate (VI)

Table 1.2 Donor atoms and corresponding ions or groups in tungsten coordination compounds.

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

acids and their salts. The polyacids are of two types: (i) isopolyacids[54], which contain only tungsten along with oxygen and hydrogen and (ii) heteropolyacids[55], which contain one or two atoms of another element in addition to tungsten, oxygen and hydrogen[56].

In pentavalent tungsten complexes the units W^{5+} and WO^{3+} also exist, but in addition dimeric structures can be found containing the $W_2O_4^{2+}$ unit[1]. Tetravalent tungsten complexes contain the structural units W^{4+}, WO^{2+} or dimeric configuration having W=W double bonds, as well as trinuclear clusters with three W atoms bonded together in a triangular configuration. Most complexes of trivalent tungsten are dinuclear such as in the $[W_2Cl_4]_n^{3-}$ ion with some of them having a triple bond between the two tungsten atoms.

All known tungstoenzymes have two pyranopterindithiolate ligands (Fig. 1.1)[9-11,13,26]. Chemical and EXAFS studies suggest that WdO, WdS, and/or WsSH groups may also be

present[57-59]. Interest in modeling the enzymes continued unabated[10,60] since the findings that tungsten-containing oxotransferase and hydroxylase enzymes posses a universal pterin dithiolene cofactor[9,11,13,61] above. Several synthetic chemical analogues of tungstoenzymes with these terminal functions have been prepared and investigated[9-11,26]. A dioxodithiolato- tungsten complex $[Net_4]_2[W^{VI}O_2(ndt)_2]$. H_2O (ndt = 2,3-naphthalenedithiolate) has been synthesized and structurally characterized[62,63]. These developments have added new significance to the chemistry of the element (Fig. 1.1).

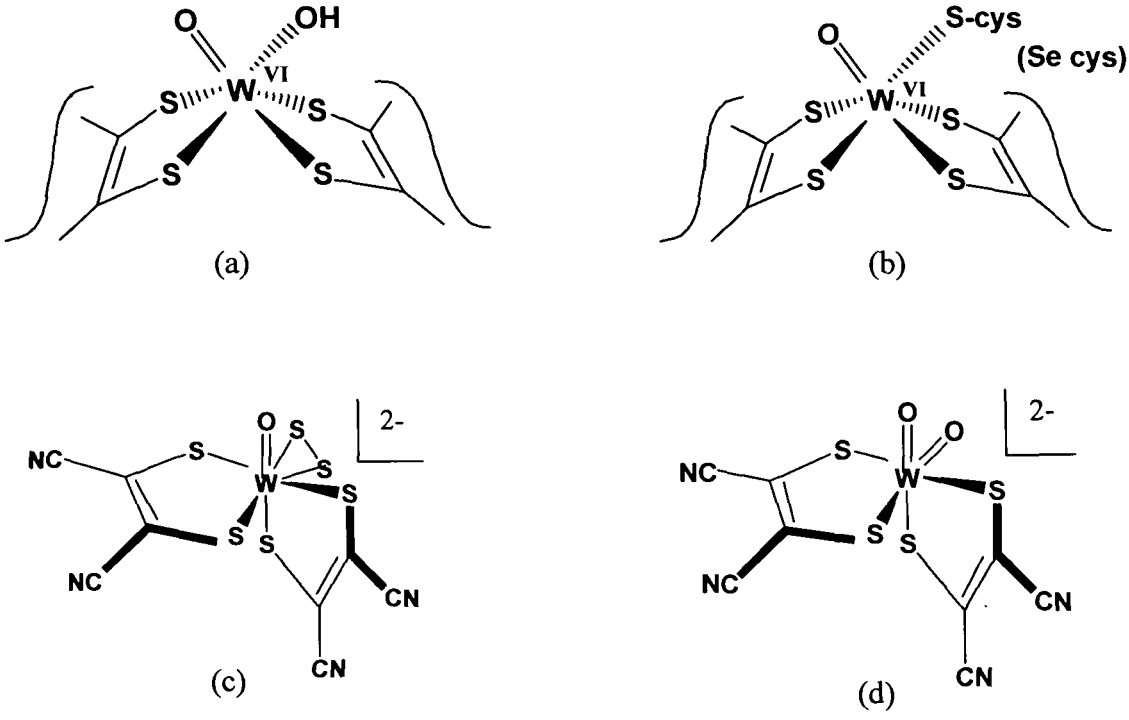


Fig. 1.1 Active-site structures of tungsten-containing enzymes, (a) aldehyde:ferredoxin oxidoreductase family[12] and (b) formate dehydrogenase family[12], and their structural functional models[10], (c) $[W^{VI}O(S_2)(mnt)_2]^{2-}$ and (d) $[W^{VI}O_2(mnt)_2]^{2-}$, respectively. Where mnt = maleonitrile dithiolate.

One of the most interesting aspects of tungsten chemistry, which has been receiving continued attention over the past decades, is their peroxo chemistry[45-51,64-67].

1.4 METAL-PEROXO COMPLEXES : SALIENT FEATURES

Peroxo-transition metal complexes, in general, have received continued attention over several years because of their important roles in biological processes[45,68-70] and in catalytic oxidations[49,70-78]. The biochemical significance of peroxo metal complexes has been emphasized in literature[42,68,69]. The reactivity of peroxides[73,74,79,80,81] and the lability of metal-oxygen bonds in special heteroligand environments in solutions are of particular *interest to biochemistry although not easy to measure directly.*

According to the rationalization made by Vaska[82], transition metal peroxides involve co-valently bound dioxygen resembling O_2^{2-} in peroxo configuration. A common characteristic of these complexes is the O-O distance, which occurs between 1.4 and 1.52 A^o (1.49 for O_2^{2-}), and the corresponding infrared frequency ν(O-O), which lies between 800 and 950 cm^{-1}.

Simple peroxo compounds of transition metals are the ones, which contain peroxides, hydroperoxides and water molecules. Whereas heteroligand peroxo compounds, a term introduced by C. Djordjevic[69], refer to metal complexes containing one to three co-ordinated peroxo groups and one or more ancillary ligands. Heteroligands may range from monodentate ions to bulky porphyrins[45,49,64,69,70,83] (F^-, Cl^-, NH_3, SO_4^{2-}, $C_2O_4^{2-}$, CO_3^{2-}, NTA, EDTA, bipy, o-phen, oxine, porphyrins, pyridine-2,6-dicarboxylic acid etc.).

The electron rich O_2^{2-} ion, owing to the presence of two extra electrons in the antibonding $O_{p\pi^*}$ orbitals, preferably forms complexes with metal ion of low d^n including d^o, and also f^o electronic configurations. The metal peroxo bonds in peroxometallates are described by σ-interactions between the metal d_{xy} orbital and an in-plane peroxo π^* orbital as suggested from *ab initio* calculations and semi empirical computations[79]. In

case of diperoxo complexes the metal $d_{x^2-y^2}$ orbital interacts with π^* orbital of the second peroxo ligand to form the metal peroxo bond.

The way in which peroxo group is expected to co-ordinate to metals can range from symmetrical bidentate to a side-on monodentate position, including all possible angles in between them. The structural classification of dioxygen complexes, rationalized by Vaska[82] can be represented as shown in Fig. 1.2. Although the term molecular oxygen refers only to the free uncoordinated O_2 molecule with the ground state configuration $^3\Sigma g$, the term dioxygen has been used as a generic designation for O_2 moiety in any of its

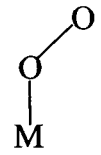
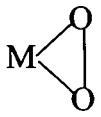
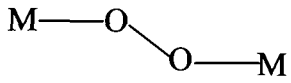
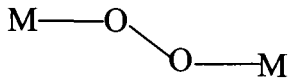
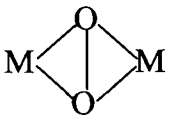
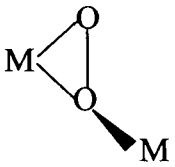
Structural type	Structural designation	Vaska classification
	η^1 dioxygen	Type a (superoxo)
	η^2 dioxygen	Type IIa (peroxo)
	η^1 : η^1 dioxygen	Type Ib (superoxo)
	η^1 : η^1 dioxygen	Type IIb (peroxo)
	η^2 : η^2 dioxygen	—
	η^1 : η^2 dioxygen	—

Fig. 1.2 Structural classification of metal-dioxygen complexes[82].

several forms and can be referred to O_2 in either a free or combined state[82]. For use of this term it is essential that a covalent bond exists between the oxygen atoms. Thus a metal dioxygen complex refers to a metal containing O_2 group co-ordinated to the metal center, and no distinction is made between neutral dioxygen in any of its reduced forms.

The bridging peroxo could vary from cis-planar and trans-planar to trans-nonplanar configuration. An unusual symmetrical double bridging was also found[84,85]. Deviations from the ideal symmetry are also observed very often[70,86]. In case of heteroligand fields they are due to the inherent symmetry of different donor atoms. Additional $p\pi^*$ electron delocalisation to the metal ion is anticipated, which could therefore favour d^o/f^o or low d^n metal ion configuration. The stereochemical polyhedra in heteroligand peroxo complexes are often fairly predictable. In oxoperoxo heteroligand surrounding, the pentagonal bipyramidal arrangement is most common for transition metal complexes, usually with two co-ordinated peroxo groups in cis- position.

Infrared spectroscopy is essential for the characterization of peroxo metal compounds. Coordination of peroxide in a side-on bidentate fashion creates a local C_{2v} symmetry which has three IR active modes[87], symmetric O-O stretching, symmetric metal-peroxo stretching, and antisymmetric metal-peroxo stretching which occur at approximately 880, 600 and 500 cm^{-1}, respectively[45]. The ν_S(O-O) is the most sensitive and intense one. All the three IR active modes are also Raman active and thus the results of Raman spectral studies not only complement the IR results but also augment them. Symmetric O-O stretching observed at approximately 850 cm^{-1} in IR is weak in case of bridging peroxide because of its very weak dipole, but it shows strong absorption in Raman spectroscopy.

The stability of peroxo complexes is generally enhanced by specific heteroligand combinations. Many simple metal peroxides often explode spontaneously, some are sensitive to shock or decompose above 0 °C, several do not exist at all as stoichiometric compounds[38] but many heteroligand peroxo complexes, on the other hand, survive recrystallization from boiling aqueous solutions, heating in vacuo, and remain unchanged for prolonged periods in closed containers[69,88,89]. The metals, Sc, Ti, V, Cr, Y, Zr, Nb, Mo, La, Hf, Ta, W[69] and U[90] form stable heteroligand peroxo complexes.

1.5 PEROXO COMPOUNDS OF TUNGSTEN – CHEMISTRY AND IMPORTANCE

It has been known for over a century that characteristic colour reaction may take place when hydrogen peroxide is added to solutions of transition metal derivatives[91] and many peroxo transition-metal compounds have been isolated in the solid state[45,49,69,91]. Besides their scientific significance, such systems are attractive as potential catalysts in biological[32,40,65,92,93] and industrial processes or their simple models[67,73,74,94-98]. 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[99,100].

Among the various d^0 transition metal peroxo systems, V(V), Mo(VI) and W(VI) derivatives attract continuous research attention because of their versatility and selectivity as organic oxidants[45,49,73,74]. Knowledge regarding the active involvement of peroxovanadium compounds in haloperoxidases[101-103], their enzyme inhibitory[102], antineoplastic[104] and insulino-mimetic properties[92,93,105-113] as well as their potent catalytic properties in the oxidation of organic and inorganic substrates[45,74,94-98,103,114] have

intensified interest in these complexes. The peroxo tungsten complexes, on the other hand, have been for the past several years object of investigation, mainly due to their application as an important class of stoichiometric or catalytic oxidizing and oxo-transfer agent in a variety of organic oxidations[46-51,115-118]. Various synthetic approaches have been developed for the oxidations of alkenes and allylic alcohols to corresponding epoxides[119-121], primary and secondary alcohols to the aldehydes and ketones[122-124], aldehydes to esters[125,126], sulfides to sulfoxides and sulfones[116,117,127] as well as hydroxylations of alkanes and arenes[74,128](Fig. 1.3). A perusal of literature however, shows that peroxotungsten chemistry has received relatively less attention compared to peroxomolybdates[49,129] in spite of the observation that pW compounds were more efficient oxidants compared to the Mo containing analogues[49,73].

There is a clear structural and isoelectronic relationship between peroxo complexes of Group 5 and Group 7 metals, which makes it worthwhile and convenient to study some of their features in parallel. Both vanadium as well as tungsten-hydrogen peroxide systems appear to be complicated owing to the formation of a number of different complexes in solution with a small change in pH of the reaction medium[67,91]. The composition of peroxo species of these metals formed in aqueous solution is sensitive to various factors viz., metal and hydrogen peroxide concentration, pH, ionic strength, and reaction temperature[45,49,67,130].

Peroxotungstate(pW) species formed in aqueous solution have been studied by several techniques including ^{17}O-NMR spectroscopy[79,131], ^{183}W NMR[132-134], Raman spectroscopy[135,136] and by electrospray ionization mass spectrometry (ESI-MS)[137,138]. Moreover, structures of tungsten peroxo derivatives are also being theoretically investigated[138]. Because of the low receptivity of the ^{183}W nucleus, ^{183}W – NMR has been of limited use[136].

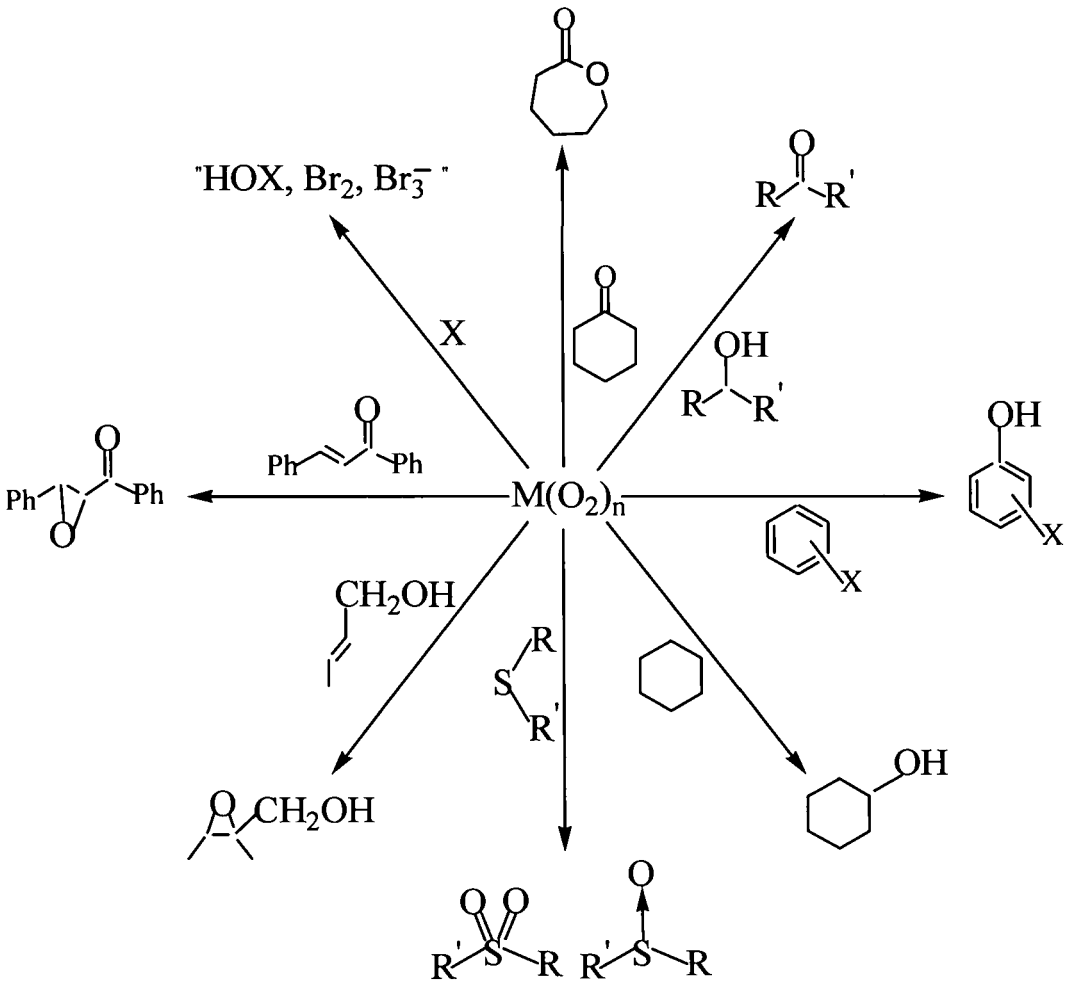


Fig. 1.3 Selected oxidations of organic compounds by peroxometal complexes[139]. M= V or W

The tetraperoxotungstate anion $[W(O_2)_4]^{2-}$ is the principal species in an alkaline solution (pH 7-9) of WO_4^{2-} and excess H_2O_2. Several salts of the yellow coloured anion have been reported[91] all of which are unstable and prone to explode. As the pH of peroxide-rich solutions of tungstate is lowered to <5 tetraperoxoanions are converted into dimers of diperoxo species[49].

$$2[W(O_2)_4]^{2-} + 5H_2O \rightleftharpoons [W_2O_3(O_2)_4(H_2O)_2]^{2-} + 2OH^- + 4H_2O_2$$

Formation of the monomeric diperoxotungsten species $[WO(O_2)_2(H_2O)_2]$ is favoured in dilute (*ca.* 0.1 mM) acidic solution (0.10-1.00 M, H^+)[140]. Several oxodiperoxotungsten(VI) complexes in different heteroligand environment have been reported[49-51,141-145] and in some cases structurally characterized (Table 1.3). In all the structurally characterized complexes of the type, $[MO(O_2)_2L_{ax}L_{eq}]$,[0/1-/2-] the metal atoms are seven coordinated, with a pentagonal bipyramidal geometry[49]. The oxo and the two η^2-peroxo groups occupy mutually cis -positions with one L ligand in axial position and the remaining L being equatorial (Table 1.3). Ligands L may also function as bridging groups in dinuclear species.

Table 1.3 Some structurally characterized monomeric and dimeric oxodiperoxo complexes of tungsten(VI)[49]

Stoichiometry	L_{ax}	L_{eq}	n	Ref.
$[WO(O_2)L_{ax}(Leq)_3]^{n-}$	F^-	F^-	2-	146
$[WO(O_2)_2L_{ax}L_{eq}]$,[0/1-/2-]	H_2O	hmpt	0	147
	H_2O	$(tacn)WO_3$	0	148
	½(ox)	½(ox)	2-	146
$[WO(O_2)_2L_{ax}L_{eq}]_2^{2-}$	H_2O	H_2O		
	¼(μ_4-ox)	¼(μ_4-ox)	2-	149

The pertungstate- hydrogen peroxide mixture has been used for oxidation of a variety of alkenes such as isolated double bonds[150,151], allylic and homoallylic alcohols[152] and α,β-unsaturated acids[46] and are considered to be the best transition metal catalyst for epoxidation reactions of alkenes with hydrogen peroxide. The combination of WO_4^{2-} / H_2O_2 is used in industry for the preparation of epichlorohydrin[98], the major raw material used in the manufacture of epoxy and phenoxy resins. Pertungstic acid and pertungstates are known to give highly stable aqueous solutions and the tungstate ion has been shown to be quite superior to molybdate and vanadate, since the transition metal ion induced decomposition of hydrogen peroxide is much slower and allows the use of a boarder pH range[153,154] of 6-7.

In 1969, Mimoun et al.[155] introduced a series of neutral seven-coordinated peroxo complexes of Mo and W, $[MO(O_2)_2Lx]$ (L = py, hmpa, dmf, H_2O and so on; x =1,2), as an important class of stoichiometric oxidants for organic oxidations. Because of their higher solubility in polar and non polar solvents, the neutral and appropriate salts of mono anions of peroxo tungsten complexes have been widely used as both stoichiometric and catalytic oxidants in oxygen-transfer reactions including oxidation of primary and secondary alcohols to aldehydes and ketones, respectively[51,156], epoxidation of alkenes[49,129], sulfides and sulfoxides to sulfoxides and sulfones[157,158], indoles, furans, organoboranes, metal alkyls (Fig.1.3). Although in majority of these investigations $[MoO(O_2)_2(hmpt)]$ has been the complex of choice, but studies indicate that the diperoxo species $WO(O_2)_2$HMPA is a more effective oxidant than $MoO(O_2)_2$HMPA in alkene epoxidation[49,129].

A significant contribution to the existing wealth of organic oxidants is the 'Venturello's complex' $(R_4N)_3[(PO_4)W_4O_4(O_2)_8]$ isolated and characterized structurally by Venturello and co-workers[115]. Such complexes, with H_2O_2 as co-oxidant, catalyze the

oxidations of a wide variety of organic substrates, normally in biphasic solvent systems with phase transfer agents. The reactivity of the species is believed to result from the presence of pairs of bridging peroxo ligands[115]. Tungsten catalysts of this type use H_2O_2 more efficiently than many other epoxidation catalysts, insofar as their unique chemistry favours oxygen transfer over peroxide disproportionation[153,154].

Quite exciting is to draw attention to an important development in the area of green chemistry is the work of R. Noyori[116]. In continuation of his design of routes to greener synthesis, Noyori described oxidation of various organic compounds using H_2O_2, a physiologically harmless tungstate catalytic system consisting of Na_2WO_4 and $Q^+HSO_4^-$ (methyltrioctylammonium hydrogensulfate) as PTC (a phase transfer catalyst) without any organic solvents and halides[116,-118,159,160].

The influence of ligation on the reactivity of peroxide complexes of V(V), Mo(VI), W(VI), and other transition metal ions is a topic of much interest. The nature of the coordinating ligand and the solvent system are very important factors on which the oxidative reactivity of peroxotungstate[139] complexes depend[161]. An increase of electron density on the metal brought in by the co-ligands would reduce the electrophilicity of peroxo complexes and also their ability to act as oxidant[81]. The activity of peroxotungsten complexes as catalysts have been fine-tuned with ligands and various correlations have been made involving the electronic and other properties of the ligand[81,161-164]. The mechanism of oxidation reactions mediated by peroxotungstates as electrophilic or radical oxidants have been studied extensively[45,74,164].

Besides the oxidations of organic substrates, the peroxo tungsten system has been reported to catalyze the oxidation of bromide in acidic medium[165]. The oxidation of bromide with peroxo-metal systems is of particular interest as such a process is actually a chemical model of the activity of vanadium-dependent haloperoxidases[45,101].

Haloperoxidases are enzymes that catalyze the two-electron oxidation of halide (X^-) by peroxide to the corresponding halogenating species X_2, X_3^- or hypohalous acid, which halogenates organic substrates RH[45,101,166,167].

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

The primary oxidized intermediate is still not known although for bromide it is equivalent of hypobromous acid, bromine, tribromide or an enzyme-bound bromonium ion-type species[45,101,166,167]. They are referred to as chloroperoxidases, bromoperoxidases or iodoperoxidases depending on the most electronegative halogen they can oxidize.

Bromoperoxidases, are involved in the biosynthesis of many brominated marine natural products ranging from simple hydrocarbons to halogenated terpenes, indoles, phenols, which often have important biological and pharmacological activity[168]. 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[45,168]. The disproportionation reaction of hydrogen peroxide is a bromide-catalyzed process.

Crystal structures of some haloperoxidase proteins *Curvularis inequalis*[169], *Ascophyllum nodosum*[170], *Corallina officinalis*[171] 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 phosphatase[172]. 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[173] 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. The biomimetic functional models reported in the literature are mostly based on monoperoxovanadium[174] or triperoxodivanadium species[101,168,175,177]. Schiff-base complexes of V(V), aqueous solution of cis-dioxovanadium(V) (VO_2^+) in acidic medium, a V_2O_5 and H_2O_2 system, KBr in excess H_2O_2 in presence of vanadyl sulphate in phosphate buffer were all found to be effective in bromination of organic substrates and were studied in detail as functional mimic of the enzyme. Thus, 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.

Concomitant with the biochemical interest on the activity of V-BPO there have been efforts to develop catalytic protocols with synthetic V-BPO mimics[77,178]. Apart from vanadate and peroxovanadate, a few other transition metal systems such as $MeReO_3$[179], MoO(oxalate)[180], MoO_3(aq)[165] and WO_3(aq)[165], catalyze the oxidation of bromide by hydrogen peroxide and are thus treated as functional mimics of V-BPO[181]. A tungstate-exchanged layered double hydroxide has also been studied as a heterogeneous catalyst in oxidative bromination of olefins by H_2O_2 system[78,182].

Conventional bromination methods involve elemental bromine, which is a pollutant and a health hazard. There is a need for benign catalytic systems, which can mimic the biological bromoperoxidase in the synthesis of brominated organics[178].

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[45] most of the model complexes were found to be catalytically active only in acid medium. It is worth mentioning here that, success has been achieved by other workers of the laboratory where the present work has been carried out, in synthesizing a series of pV compounds with the distinctive feature of having a μ-peroxo group of the type $[V_2O_2(O_2)_3.(L)_3].H_2O$ (L = amino acid or dipeptide)[130,183], as well as a polymer-anchord peroxovanadate compound[184,185] which could act as powerful oxidants of bromide with good activity at physiological pH thus mimicking the enzyme V-BPO. The μ-peroxo vanadium compounds however, undergo rapid degradation in solution with loss of its high bromination activity[130]. Moreover, a set of dinuclear and mononuclear pW compounds with amino acids and peptides as auxiliary ligands were proved to be efficient oxidants of bromide at neutral pH.

However, a great deal of effort is still required to develop peroxometallates with definite potential for application as safer alternative synthetic catalyst for organic bromination reactions. Much remains yet to be explored on activity of well-defined pW compounds in oxidative bromination.

In addition to halide oxidation, the haloperoxidases and some of their model compounds are capable of oxidizing organic sulfides to sulfoxides[186-188]. The selective oxidation of organic sulfides to sulfoxides is an attractive and important method in organic chemistry, especially because of the potential use of sulfoxides as useful building blocks for construction of many chemically and biologically active molecules including therapeutic agents such as antiulcer, antibacterial, antifungal, and antihypertensive[189-196]. Moreover, research on oxidative desulfurization (ODS), as a method for removal of sulfur from fuels, and industrial products and wastes, has been growing and appeared to

be promising[197-201]. Thus, selective oxidation of sulfide has received continued attention leading to the development of a number of useful reagents[201-204], acids[205-207], transition metal based catalysts, including vanadium[208,209], rhenium[210], iron[211,212], manganese[213,214], titanium[215,216] and tungsten[48,159,217-221] based systems. While all these are important progresses, there are several limitations associated with the protocols being used in practice viz., non-selectivity, over oxidation, high cost and toxicity of the catalyst. Moreover, most of these procedures require chlorohydrocarbon solvents that affect human health and environment. Recently, Noyori et al.[159] have shown that oxidation of sulfides to sulfoxide and sulfone with H_2O_2 could be achieved by using Na_2WO_4 under solvent and halide free conditions. A phosphonic acid promoter and an acidic quaternary ammonium salt, $[CH_3(n\text{-}C_8H_{17})_3N]HSO_4$, are used along with the catalyst. However, the protocol needs homogeneous reaction conditions and therefore the catalyst could not be recovered for further cycles.

The use of solid catalysts under heterogeneous conditions, which allows easy separation of the catalyst from the reaction mixture are considered ideal for such reactions. In recent years, selective oxidation of sulfide has been carried out with a large number of supported reagents and catalysts[222-224]. Gomez et al.[202] conducted a comparative study with different oxidant and support to delineate the role of support in selectivity of sulfoxidation, according to which acid support (amberlyst) gave sulfoxide selectively, basic support (basic alumina) increased the proportion of sulfone formed[225]. Some of these systems however, are associated with the drawback of gradual leaching of the catalytic species during the repeated catalytic cycles. With an increasing level of environmental consciousness, there is much incentive to find new and strategically important processes using a robust and recyclable catalyst that provides higher atom utilization to minimize pollution levels using greener ingredients. It is noteworthy that in

spite of several advantages associated with immobilization of active catalyst on polymer support, there appears to be very little information available on application of peroxometal compounds supported on polymers in organic oxidations.

In addition to the importance received by the pW due to their well established role as catalytic or stoichiometric oxidant, there has been a resurgence in interest in peroxochemistry of tungsten since it has been demonstrated that tungstates and peroxotungstates (pW) present in a solution of W-H_2O_2, like vanadate and peroxovanadates (pV), mimic the insulin bioeffects in rat adipocytes[32]. Shechter and co-workers observed[32] that the higher efficacy of the pertungstates and permolybdates as insulinomimetic agents originated from their oxidizing activity relative to reduced GSH. W-H_2O_2 system was also found to be more potent inhibitors of hydrolysis of phosphoproteins[32,41,226] compared to tungstate. Significantly, a correlation was found to exist between the phosphatase inhibitory abilities of peroxo metal complexes, their abilities to promote activation of insulin receptor[42,92,174], and their insulin mimetic activities. Although a large number of heteroligand pV complexes have been tested for possible insulin like activities in recent years[227], most of the peroxo compounds are unstable under physiological condition and end with radical formation when subjected to redox processes[93,228] which limits their utility as therapeutic agents. It is therefore suprising that, despite the large number pW compounds that were synthesized in recent years and the knowledge that peroxotungstates, formed in a solution of W-H_2O_2 were stable in solution of a wide range of pH values[32], there still remains a dearth of reports dealing with pharmacological potential of discreet synthetic peroxotungsten compounds or their effect on the activity of different enzyme functions including phosphohydrolases. Studies on enzyme inhibition by metal complexes is important in the context of gaining

an insight into the mechanism of action of inorganic drugs and is an important area of current research[35,38].

It is noteworthy in this context that, a number of pV, pW and pMo compounds with biogenic co-ligands synthesized in our laboratory were found to be potent inhibitors of alkaline phosphatase[229-232]. Furthermore, immobilization of pV or pMo species on water-soluble polymers[184] not only enhanced their stability, but also altered their mode of inhibitory effect on phosphatase activity. However, a survey of literature reveals that screening of peroxo-metal incorporated soluble macromolecules for their biochemical potential have so far received scant attention.

1.6 POLYMER BOUND METAL COMPLEXES

Anchoring of an active transition metal complex to a functionalized polymer is interesting from the view point of designing effective catalysts as well as modeling of complex biomolecules and bioprocesses. 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 and waste water treatment[233-239]. In addition they are used as enzymatic models[240-245].

Various approaches to prepare 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. 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[245].

On the basis of the classification proposed by Wohler and Pomogailo a metal complex[246,247] or metal is classified by the kind of interaction it has with a macromolecule. Metal complexes or metals can be part of a macromolecular chain or network as follows:

Type I : Binding at a macromolecule

The metal part (metal ion or metal complexes) is bound to a chain of a linear or cross-linked organic polymer via a covalent (at the metal), a coordinative (at the metal) , a complex(at the ligand of a complex), an ionic or a π-bond to form the so called "Macromolecular Metal Complex" as schematically shown in Fig 1.4.

Type II: Ligand Macromolecular Complex

The ligand of a metal complex is a part of a macromolecular chain or network as shown in Fig. 1.5. In addition to the direct synthesis of macromolecular metal complexes from low molecular weight precursor, a macromolecular ligand can be prepared first and subsequently metallated in a second step.

Type III: Metal Macromolecular Complex

The metal of a metal complex or another metal derivative is directly part of a macromolecular chain or network as shown in Fig. 1.6. In most cases the metal is connected with another element such as C, N, O, S via a covalent, a coordinate, an ionic or a π-bond.

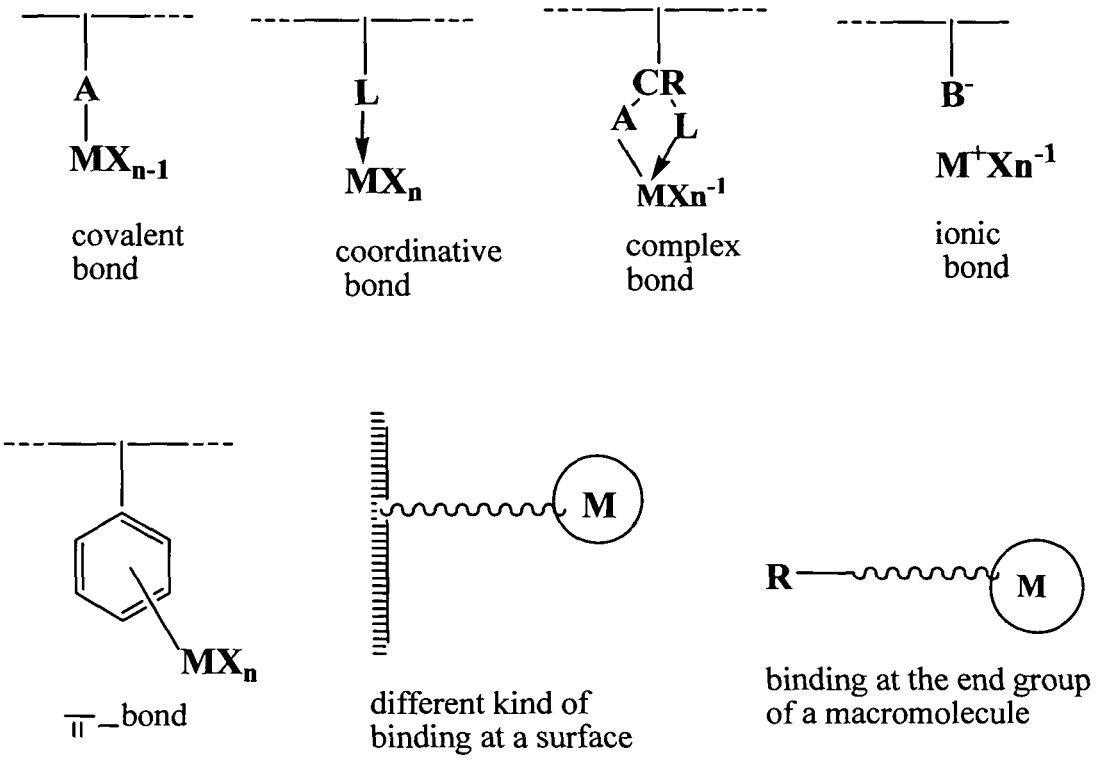


Fig. 1.4. Schematic model of binding of metal ion, metal complexes or π-π complexes at macromolecular carriers[246].

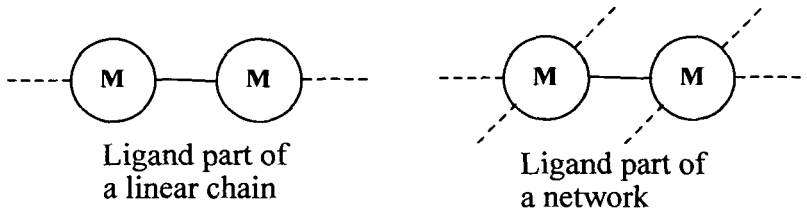


Fig. 1.5 Schematic model of ligand of a metal complex is a part of a macromolecule[246].

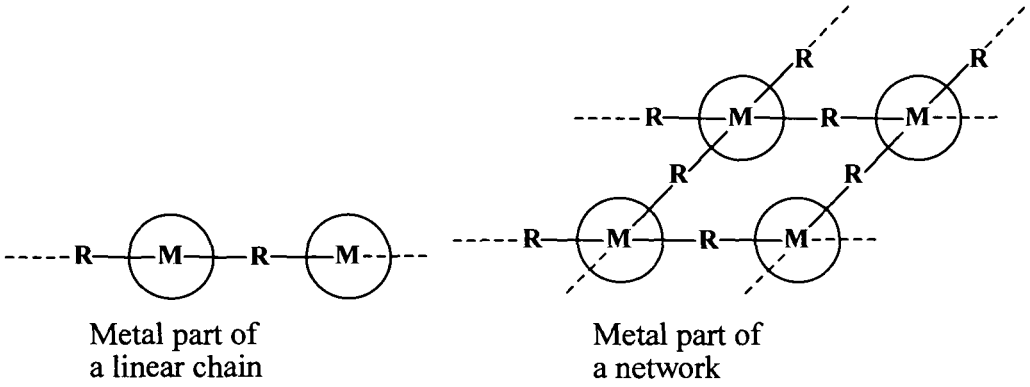


Fig. 1.6 Schematic model of metal as part of a macromolecule[246].

Type IV: Macromolecule Incorporated Metal Complexes and Metals

The physical incorporation of metal clusters or metal complexes in macromolecules (Fig. 1.7) has become an important field. By stabilization of metal clusters in macromolecular environment new composite materials have been synthesized.

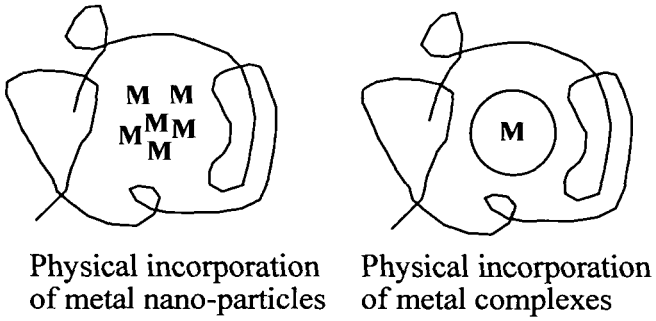


Fig. 1.7 Schematic model of physical incorporation of metal or metal complexes[246].

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 characterize.

The cross-linked copolymers of styrene with butadiene and divinylbenzene are used most extensively[248]. The choice of styrene in the polymeric back bone is favoured due to its capability of easy functionalization through the aromatic ring with different functional groups for anchoring complexes[249-254]. Thus 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)[255-257]. Ion exchange resin is also reported to be used for anchoring cationic and anionic forms of complexes[258]. Besides these, poly(vinylbenzyl)chloride and poly(glycidyl methacrylate) cross-linked with divinylbenzene or ethylene glycol dimethacrylate are commonly used as polymer-support[259].

Table 1.4 The summary of combinations of metal complexes and macroligands, as well as catalysed reactions most commonly used in practice[260].

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,Pthalocyanines	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)

1.6.1 IMMOBILIZED METAL COMPLEXES AS CATALYSTS

The catalytic properties of immobilized metal complexes are controlled by a number of factors such as the nature and distribution of attached transition metal ions and the character of the polymer support, together with unreacted functional groups of the polymer after fixation and activation of metal complexes[261]. Besides these, the cross-linking level in the polymer-support also offers different activity as well as selectivity of the anchored-metal complexes[262,263]. 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 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[261].

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. Immobilization of metal complexes usually increase their efficiency and stability[264]. In fact, immobilized catalysts combine the main features of homogeneous, heterogeneous and enzymatic catalysts.

A branch of chemically active polymers is "redox polymers"[264], 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 redactor by Jones in 1889 [264]. By 1963 redox active polymers had found application to the quantitative redox of metal ions and organic substrates[265,266] 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[264].

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[267], hydrocarbons[268,269], selective epoxidation[270-273], hydroformylation of olefins[274] and etherification[275], 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[116]. In terms of polymer supported system the most widely investigated are W(VI)/H_2O_2, V(V)/ROOH, Mo(VI)/ROOH[276]. Polymer supported Mo(VI) and V(V) are reported by Stamenova et al.[277] using ethylene-propylene rubber and cross-linked poly(ethylene oxide) grafted or interpenetrated with poly(acrylic acid), poly(methacrylic acid), poly(4-vinyl pyridine) and poly(vinyl alcohol) as the polymer support which were found to be effective catalyst in styrene epoxidation using ethylbenzene hydroperoxide as the oxidant. D. C. Sherrington and his co-workers[270,276,278,279] reported few polymer immobilized V(V), Mo(VI) and W(VI) complexes which could catalyze alkene epoxidation. They used chloromethylated poly(styrene), poly(glycidyl-methacrylate) and polybenzimidazole based chelating resins as support. These systems were applied for alkene epoxidation. More recently, M. R. Maurya et al.[280-283] reported chloromethylated-polystyrene cross linked with divinylbenzene based support for V(V), Mo(VI) and W(VI) complexes for catalysis of epoxidation of alkene as well as oxidation of sulfide. Reports are available on catalytically active O_2 bound macromolecules consisting of metals viz., Cr, Mn, Fe, Co, Mo[276]. 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[284]. In addition to Co macrocomplexes, immobilized Cu^{2+} compounds are most often used for O_2 activation[285]. Recent developments in biomimetic redox catalysis have shown that enzymes are biological polymeric catalysts which can be mimicked, and that the processes of nature can be modeled and understood.

1.6.2 MACRO COMPLEXES AS ENZYMATIC MODELS

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[240,245]. This resemblance may also be explained by the molecular mobility both of proteins and macroligands in synthetic protein analogues.

Metal polymers are interesting as models of catalase, peroxidase, proteolytic and other enzymes[286]. 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[241-243]. These are studied as model systems of metal enzymes for cytochrome P450, myoglobin and hemoglobin[241-243]. 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[264]. Enzyme catalase is an effective catalyst for H_2O_2 decomposition. There are many heterogenized enzymatic 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[244]. Catalase-type activity of Cu^{2+} complexes immobilized on CSDVB modified by Schiff bases was studied comprehensively[286].

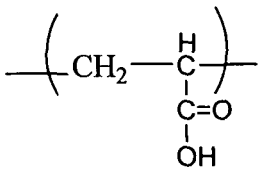
1.7 METAL COMPLEXES SUPPORTED ON SOLUBLE POLYMERS

The concept of attaching metal complexes to the soluble polymers is receiving increasing attention due to their potential application in diverse fields[287,288,289]. One of promising trends in catalyst design is the use of water soluble macromolecular metal complexes[287,289]. Development of systems based on water soluble polymers is one of the main research tasks of polymer chemistry, since for natural macromolecules, such as proteins and DNA, water is the basic solvent[287]. In the last few decades, synthetic water soluble polymers have found wide use in various biomedical applications[289-292] and separation processes[288,293-296].

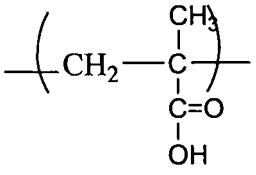
Despite the well known advantage of insoluble supports, there are several shortcomings in the use of these resins due to the heterogeneous nature of the reaction conditions[297]. The problems associated with insoluble polymer support include nonlinear kinetic behavior, unequal distribution or access to the chemical reaction, solvation problems associated with the nature of the support, and synthetic difficulties in transferring standard organic reactions to the solid phase[297]. A way to overcome this problem is to use low molecular weight soluble linear polymers as supports[297-301] which reinstated the familiar reaction conditions of classical organic chemistry[297]. In fact, transition metal complexes attached to soluble non-cross linked polymer is essentially a homogeneous catalyst with macromolecular ligands. One of the advantageous aspects of use of the soluble polymer support is that the chemical reactions performed on such

polymers can be monitored by high resolution solution phase ^{1}H and ^{13}C NMR spectroscopy[302,303]. The recovery of the polymer supported catalyst can be achieved by temperature or solvent-induced precipitation followed by filtration[297,301].

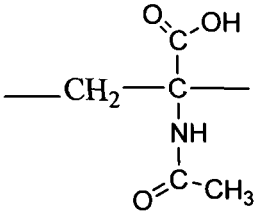
The use of soluble polymers to recover catalyst and ligands in synthetic approaches to peptide and oligopeptide synthesis were developed by Merrifield and Letsinger in the 1960's[304,305]. These discoveries revolutionised the synthesis of biomolecules[306]. They provided impetus for research in industrial 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[307]. 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 hydroformylation[309,309], wacker oxidation[310,311], hydroxylation of aromatic compounds[312] and epoxidation[313]. Polycarboxylic acid and its derivative are often used to synthesize 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 hydroformylation of 1-alkenes[314]. Polyacrylamide modified with optically active phosphine containing ligands and Rh catalyst were used for asymmetric hydrogenation of prochiral amides[315,316]. Among the first soluble macromolecular metal complexes to be used as catalyst were modified linear polystyrenes[317-320].



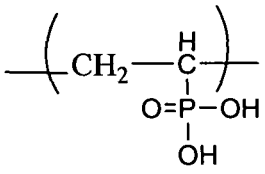
Polyacrylic acid
(PAA)



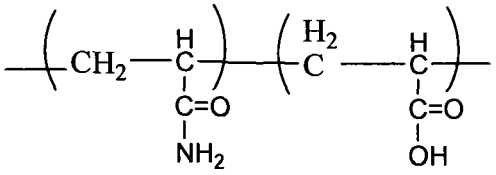
Polymethacrylic acid
(PMA)



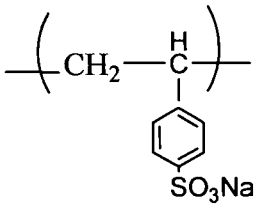
Polyacetylamino acrylic acid
(PAAA)



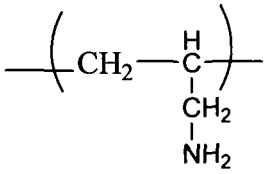
Polyvinylphosphonic acid
(PVPhA)



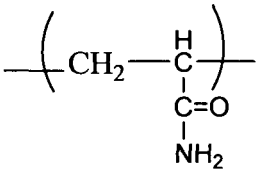
Polyacrylamide-co-acrylic acid
(PAm-co-PAA)



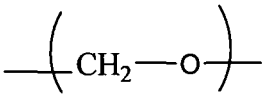
Poly(sodium 4-styrenesulfonate
(PSS)



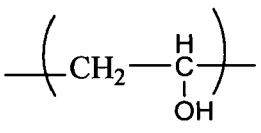
Polyallyamine
(PALA)



Polyacrylamide
(PAm)



Polyethyleneglycol
(PEG)



Polyvinylalcohol
(PVa)

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

Besides the use of soluble macromolecular metal complexes in catalysis there has been considerable contemporary interest in development of pharmaceutical formulations consisting of water soluble polymers[321-324]. A group of polymers has been used in biological systems[289] such as poly(acrylamide)[325], poly(4-vinylpyridine)[325], derivatives of poly(aspartamide)[325], poly(ethyleneimine)[325], and derivates of poly(methacrylamide) with dextrane[325], polyphosphazenes[326], derivates of poly(methacrylic acid)[327,328], synthetic poly(aminoacids)[329,330], analogues of nucleic acids[331,332], poly(ethylene oxide)[333], copolymers of vinyl pyrrolidone[334], polyamides[335], and polyamines[335]. Polymers as drug carriers have been investigated to achieve efficient delivery of the drug molecule to the targeted cell. A few linear and water soluble polymeric platinum complexes have been developed in recent years which are reported to be useful anticancer agents[336,337]. 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 behaviour in aqueous solution. For this reason they are potentially biologically active compounds. In this context, Lee and Rashidova[338] 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 behaviour, and the comparison of the biological properties between the polymer–metal complexes and the polymer bases were performed. Nandi and co-workers[339] studied the bactericide activity of metal ions and polymer–metal complexes with Co^{2+}, Zn^{2+} and Cu^{2+} whereas Nonaka and co-workers[340] 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+}. It is observed that the polymeric based anti-bacterial agents are non-volatile, chemically stable, and difficult to permeate through the skin of man or animal compared with

conventional antibacterial agents of low molecular weight compounds. So, they can reduce the loss associated with volatilization, photolytic decomposition, and transportation[289,341].

Notwithstanding the tremendous progress in the field of metal containing polymers, as well as numerous pW complexes synthesized and studied in recent years, there still remains a paucity of information pertaining to structurally defined peroxotungsten compounds anchored to polymer supports in general, and water soluble polymers in particular.

1.8 RESEARCH OBJECTIVES

From the foregoing discussion it is evident that synthesis and characterization of well defined peroxotungsten(VI) complexes attached to polymer matrices and assessment of their stability, catalytic and biochemical properties constitute a rewarding and worthwhile area of investigation.

Major objectives of the present research programme are as follows:

(i) To establish viable synthetic routes to newer peroxo complexes of tungsten(VI) in macroligand environment by anchoring peroxotungstate species to water soluble as well as insoluble polymer matrices and to characterize them.

(ii) To study the stability of the water soluble compounds towards decomposition in solution under varying pH conditions.

(iii) To explore the efficacy of the supported pW compounds as catalyst or oxidant in oxidation of organic sulfides under mild and environmentally acceptable condition.

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

(v) To explore biochemical properties of the polymer-bound pW compounds, vis-à-vis free mononuclear and dinuclear heteroligand peroxo complexes of W(VI), particularly involving their interaction with enzymes such as phosphohydrolases and catalase.

Chapters **3** to **7** of the thesis present interpretative accounts of the results of our studies on the afore mentioned aspects of peroxotungsten chemistry. Each of these Chapters has been so designed as to make it a self-contained one with brief introduction, sections on experimental, results and discussion, and conclusion followed by relevant bibliography. Most of the new results have been either published or are 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:

Sodium tungstate, potassium bromide, potassium iodide, potassium hydrogen phosphates, phenol red, acetone, diethyl ether, ethyl acetate, petroleum ether 60°- 80°C, sodium hydroxides, resorcinol, salicylaldehyde, pyrogallol, catechol, acetanilide, methanol, acetonitrile (E. Merck, Mumbai, India), dichloromethane, chloroform, 30% hydrogen peroxide (v/v) (RANKEM), poly(sodium acrylate) (M_w = 2100) (Fluka), tungstic acid, poly(acrylonitrile) (M_w = 48200), poly(sodium methacrylate) (M_w = 4000), poly(acrylamide) (M_w = 10000) and poly(sodium vinyl sulfonate) (M_w = 4000), acid phosphatase from wheat thylakoid membrane (ACP), alkaline phosphatase from rabbit intestine (ALP), p-nitrophenyl phosphate (p-NPP), catalase, methyl phenyl sulfide (MPS), dimethyl sulfide (DMS), dibutyl sulfide (DBS), butyl propyl sulfide (BPS), dibenzothiophene (DBT), phenylvinyl sulfide (PVS), 2-(phenylthio)ethanol (PTE) and allyl phenyl sulfide (APS) (Sigma-Aldrich Chemical Co., Milwaukee, USA), cysteine, sodium thiosulphate (CDH, New Delhi, India), potassium dihydrogen phosphates, aniline, nitroanilines, aminophenols, quinol, glacial acetic acid, sodium acetate and $MgCl_2$ (SD Fine Chemicals, Mumbai, India). $[WO(O_2)_2$(glycyl-glycine)$].3H_2O$ (**MWG**), $Na_2[W_2O_3(O_2)_4$(glycyl-glycine$)_2].3H_2O$ (**DWG**)

and $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ (**DWC**) were prepared by the method described earlier[1-3].

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

2.2 ELEMENTAL ANALYSES

2.2.1 Tungsten

2.2.1.1 Gravimetry[4]

Tungsten was estimated gravimetrically as $BaWO_4$ [4]. An accurately weighed amount of the water soluble compounds, **PAW (3.2)**, **PMAW (3.3)** or **PAmW (3,4)** was taken in a beaker containing 250 mL of distilled water. pH of the solution was adjusted to *ca.* 7 by adding NaOH (0.1M) and was boiled for *ca.* 30 min. To the boiling solution a saturated $BaCl_2$ solution was added dropwise with constant stirring. The precipitate formed was allowed to settle for a few minutes. The supernatant liquid was tested for complete precipitation by adding few drops of barium chloride solution. A slight excess of precipitating agent was added to ensure complete precipitation. The mixture was kept covered over a steam-bath for 1 h in order to allow time for complete precipitation of $BaWO_4$. The precipitate was then allowed to settle at room temperature and the clear supernatant liquid was again tested for complete precipitation. The digested precipitate was then filtered through a constant-weighed sintered glass crucible (Grade 4) and repeatedly washed with hot water. Subsequently, the residue was ignited at 500°C in an electric muffle furnace followed by cooling in a desiccator. The heating process was continued until constant weight was obtained.

In case of water soluble compound **PSW (3.5)**, an accurately weighted amount of the compound was initially ignited in a Bunsen flame to remove the polymer. After removal of the polymer, the residue was transferred to a beaker containing 250 mL distilled water. Subsequently, tungsten was estimated by following the procedure as mentioned above for compound **PAW (3.2)**.

In case of insoluble compound **PANW (3.1)**, an accurately weighed amount of the compound was treated with HCl (4N) to detach the anchored tungsten from the polymer matrix. The mixture was filtered and the residue containing the solid polymer was washed several times with HCl (4N) for complete removal of the metal. The solid residue was discarded and the filtrate and the washings were transferred to a 250 mL beaker. Subsequently, tungsten was estimated by following the procedure as mentioned above for compound **PAW (3.2)**.

2.2.2.2 EDX analysis

Tungsten content was also determined by Energy Dispersive X-Ray (EDX) analysis.

2.2.2 Peroxide[5-7]

2.2.2.1 Permanganometry[5]

An accurately weighed amount of a peroxotungstate 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 peroxotungsten(VI) compounds.

2.2.2.2 Iodometry[6]

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 peroxotungstate(VI) 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 present in the compound.

2.2.2.3 By standard Ce(IV) solution[7]

An accurately weighed amount of a peroxotungstate(VI) compound was dissolved in a 0.7N sulphuric acid solution in the presence of an excess of boric acid. Peroxide was then determined by titrating with a standard Ce(IV) solution.

2.2.3 Carbon, Hydrogen and Nitrogen

The compounds were analyzed for carbon, hydrogen and nitrogen by Perkin Elmer 2400 series II at the Department of Chemical Sciences, Tezpur University.

Carbon, hydrogen and nitrogen content was also obtained from EDX analysis.

2.2.4 Sodium

Sodium contents were determined by EDX analysis.

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 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 in 1 cm quartz cuvettes. All the absorbance values are denoted as, e.g., A_{592}, A_{340} at the wavelengths indicated.

2.3.3 Infrared (IR) spectra

The infrared (IR) spectra were recorded with samples as KBr pellets in a Nicolet model impact 410 FTIR spectrophotometer.

2.3.4 Surface morphology analysis by Scanning Electron Microscope

The SEM characterization was carried out by using the JEOL JSM-6390LV Scanning Electron Micrograph attached with energy dispersive X-ray detector. Scanning was done at 1–20 μM range and images were taken at a magnification of 15–20 kV. Data were obtained using INCA software. The standardization of the data analysis is an integral part of SEM-EDX instrument employed.

2.3.5 ^{1}H-NMR spectra

The ^{1}H-NMR spectra were recorded in deuterated chloroform in JEOL JNM-ECS400 spectrophotometer. 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.

2.3.6 ^{13}C-NMR spectra

The ^{13}C-NMR spectra were recorded in a JEOL JNM-ECS400 spectrometer at carbon frequency 100.5 MHz, 1,31,072 X-resolution points, number of scans 8000, 1.04 s of acquisition time and 2.0 s of relaxation delay with ^{1}H decoupling method. The spectra were recorded in D_2O or {(DMSO-d + DMF) (1:4)} for the newly synthesized polymer bound water soluble or insoluble pW compound, respectively. The spectra of organic sulfides, sulfoxides and sulfones were recorded in $CDCl_3$ as solvent.

2.3.7 GC analysis

GC analysis was carried out on a CIC, Gas Chromatograph model 2010 using a SE-52 packed column (length 2 m, 1/8'' OD) with a Flame Ionization Detector (FID), and nitrogen as carrier gas (30 mL/min).

2.3.8 HPLC analysis

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

2.3.9 Thermogravimetric analysis

Thermo gravimetric analysis was done in SHIMADZU TGA-50 system at a heating rate of 10 °C/min under an atmosphere of nitrogen using aluminium pan.

2.3.10 Melting point determination

Melting points were determined in open capillary tubes on a Büchi Melting Point B-540 apparatus and are uncorrected.

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CHAPTER 3

Polymer Anchored Peroxo Complexes of Tungsten(VI): Synthesis, Characterization and Stability in Solution

3.1 INTRODUCTION

The importance of peroxotungsten (pW) compounds, which rendered them the focus of one of the active areas of contemporary research, have been emphasized in the literature[1-4] and highlighted in the introductory Chapter. Our interest in the design, synthesis and study of pW compounds has been spurred by the importance of pW systems mainly attributable to their catalytic potential[1,5,6], as well as the increasing awareness of the biochemical relevance of the metal and its compounds[7-9]. Although numerous reports deal with the synthesis and reactivity of heteroligand peroxotungsten(VI) compounds[1,10-14], information on structurally-defined peroxotungstates where a macromolecule such as a functionalized polymer provides the heteroligand environment is scanty[15-18].

Metal containing macromolecules in general, constitute a fascinating field of science[19,20]. It is readily understandable that materials with unusual properties are obtained by having a metal as part of a macromolecule[21]. Apart from the insoluble cross-linked polymers, the utility of water soluble polymers as supports in organic chemistry and biology is increasingly being recognized in recent years[22,23].

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[22,23]. Binding of active drug molecules

including low molecular weight metal complexes to soluble macromolecular carriers is of 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[24-27]. Polymers often lack many inconvenient properties of monomeric species, such as lability, volatility, toxicity and odour[28].

It is pertinent to mention that other workers of our research group successfully synthesized series of stable and well-defined macrocomplexes by incorporating peroxo vanadium (pV) as well as, peroxomolybdenum (pMo) species into water soluble polymer matrices[29,30]. To the best of our knowledge, these are the first examples of peroxometallates attached to water soluble polymers. Each of these macro complexes exhibited unique biochemical as well as oxidant properties[29,30]. There is an obvious need to obtain this class of new materials preferably using easy methods of synthesis. Anchoring of peroxometallates to water soluble polymers has recently been recognized as a promising new field[31].

In the present work therefore, we have focused on establishing viable synthetic routes to new pW systems, supported on soluble as well as insoluble polymer matrices. Our effort has been directed towards two goals: (i) to gain an access to biomimetic catalysts or reagents for organic oxidations under mild conditions, and (ii) to generate peroxometallates with biologically important characteristics.

A polymeric support is likely to impart stability to the anchored complex species. Proper choice of the functional group is an important prerequisite in order to obtain a stable polymer-metal linkage[28,30,32]. The polymeric ligands chosen for the present study required to possess the ability to form stable complexes with peroxo-tungsten(VI) moiety.

The information derived from synthesis of unbound heteroligand peroxotungstates have been particularly useful in identifying co-ordinating groups which will help in retaining the anchored complex species after completion of the reaction cycles.

We selected, for the purpose of this investigation, polymers viz., poly(acrylate), poly(methacrylate), poly(acrylamide) and poly(vinylsulfonate), as supports owing to the advantageous features associated with these polymeric species such as their convenient method of preparation or commercial availability, water solubility, chemical stability, presence of appropriate functional groups for easy attachment of active metal complexes. These 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 sulfonic acid based polymers and their derivatives[33-36]. The insoluble polymer PAN, was chosen for immobilization of the pW species because, apart from being a non-toxic, cheap and commercially available reagent acrylonitrile polymers have attracted much attention for their application in diverse areas that include medicine[37], antioxidants[37], surface coatings[38], catalysis[39], textiles treatment[37], binders[37] and as adsorbant for removal of heavy metal ions from water[40,41]. As far as we are aware, neither PAN nor any of the known soluble polymers have been explored till date, for use as polymeric support to obtain peroxotungsten species in macro ligand environment.

The present chapter reports the synthesis and characterization of a series of new water soluble polymer bound diperoxotungsten complexes of the type **[$WO(O_2)_2$(carboxylate)]–PA [PA = poly(sodium acrylate)] (PAW) (3.2), [$WO(O_2)_2$(carboxylate)]–PMA [PMA = poly(sodium methacrylate)] (PMAW) (3.3), [$WO(O_2)_2$(amide)]–PAm [PAm = poly(acrylamide)] (PAmW) (3.4),** and

$[WO(O_2)_2$(sulfonate)]–PS [PS = poly(sodium vinyl sulfonate)] (PSW) (3.5), and an insoluble polymer immobilized monoperoxotungsten compound, **$[WO_2(O_2)(CN)_2]$—PAN [PAN = poly(acrylonitrile)] (PANW) (3.1)**. The results of studies on stability of the compounds toward decomposition in solution are also reported in this chapter.

3.2 EXPERIMENTAL SECTION

3.2.1 Synthesis of $[WO_2(O_2)(CN)_2]$—PAN [PAN = poly(acrylonitrile)] (PANW) (3.1)

Solid H_2WO_4 (1.17 g, 4.72 mmol) was dissolved in minimum volume of H_2O_2 (30% solution, 10 mL, 88.2 mmol) in a 250 mL beaker with constant stirring at room temperature. The pH of the clear solution obtained was recorded to be 1.21. Concentrated sodium hydroxide (*ca.* 8 M) was then added to the above solution dropwise with constant stirring to raise the pH of the reaction medium to 5.0. Keeping the temperature of the reaction mixture below 4 °C in an ice bath, 1.0 g of poly(acrylonitrile) was added to it. The suspended polymer beads were allowed to swell in the reaction mixture under continuous stirring for 24 h. The supernatant liquid was then decanted and the white residue was repeatedly washed with pre-cooled acetone. The product was separated by centrifugation and dried *in vacuo* over concentrated sulfuric acid. In the solid state the compound was found to be stable for several weeks stored dry in closed containers at ≤30 °C.

3.2.2 Synthesis of $[WO(O_2)_2(carboxylate)]$–PA (PA = poly(sodium acrylate) (PAW) (3.2), $[WO(O_2)_2(carboxylate)]$–PMA [PMA = poly(sodium methacrylate)] (PMAW) (3.3), $[WO(O_2)_2(amide)]$–PAm [PAm = poly(acrylamide)] (PAmW) (3.4) and $[WO(O_2)_2(sulfonate)]$–PS [PS = poly(sodium vinyl sulfonate)] (PSW) (3.5)

The procedure adopted for the synthesis is common to all the four water soluble macromolecular complexes. In a typical reaction, H_2WO_4 (1.0 g, 4.0 mmol for compound **3.2** and **3.3**; 5.28 g, 21.12 mmol for **3.4**; 2.88 g, 11.53 mmol for **3.5**) was dissolved in minimum volume of 30% H_2O_2 (10.0 mL, 88.2 mmol for **3.2** and **3.3**; 52.8 mL, 465.69 mmol for **3.4**; 28.8 mL, 254.11 mmol for **3.5**) in a 250 mL beaker with constant stirring at room temperature (*ca.* 30 °C). The pH of the clear solution obtained was recorded to be 1.21. Concentrated sodium hydroxide (*ca.* 8 M) was then added to the above solution dropwise with constant stirring to raise the pH of the reaction medium to 5.0. Keeping the temperature of the reaction mixture below 4 °C in an ice bath, 1.5 g of the respective polymer was added to it. The reaction mixture was kept for 24 h under continuous stirring at a temperature below 4 °C to provide sufficient contact time for interaction of reactants. On adding pre-cooled acetone (*ca.* 50 mL) to this mixture under vigorous stirring, a white pasty mass separated out. After being allowed to stand for about 30 min, the supernatant liquid was decanted and the residue was treated repeatedly with acetone under scratching. The microcrystalline product was separated by centrifugation, washed with cold acetone and dried *in vacuo* over concentrated sulfuric acid. The compounds were subsequently dried by heating upto 70 °C under nitrogen atmosphere. In the solid state these compounds were found to be stable for several weeks stored dry in closed containers at ≤30 °C.

3.2.3 Elemental analysis

Quantitative estimations of tungsten, 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, TG analysis as well as scanning electron micrographs (SEM) and EDX analysis were done as per methods described in Chapter **2**. Structurally significant IR bands and their assignments are reported in Table 3.2. In Table 3.4, TGA data of the complexes are reported. Table 3.3 consists of ^{13}C NMR chemical shift values for the complexes and their respective free polymers.

3.2.5 Stability of the complexes in solution

Stability of the compounds in distilled water, at their natural pH, was studied by determining the peroxide content in aliquots drawn from the respective solution of the compound containing **PAW (3.2)** (0.122 mg/mL) or **PMAW (3.3)** (0.160 mg/mL) or **PAmW (3.4)** (0.156 mg/mL) or **PSW (3.5)** (0.085 mg/mL) at different interval of time by the method described in Chapter 2. The initial peroxide concentration in each of the test solutions was maintained at 0.4 mM. As a measure of stability of the compounds in solution changes in absorbance of their electronic spectral band at *ca.* 230-250 nm at ambient temperature were recorded at 30 min gap for a period of 12 h. Stability of the compounds in solution at pH 1.2 and 2.1 (50 mM KCl/HCl buffer), 3.1 (50 mM citrate buffer), and 4.4, 7.0 or 8.0 (50 mM phosphate buffer) was measured similarly.

3.3 RESULTS AND DISCUSSION

3.3.1 Synthesis and characterization

3.3.1.1 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. In the present study, it has been possible to isolate two types of peroxotungstate species viz., dioxomonoperoxotungsten(VI) (**3.1**) immobilized on an insoluble polymer matrix, PAN and oxodiperoxotungsten(VI) complexes (**3.2-3.5**) supported on water soluble macromolecules possessing pendant functional groups such as carboxylate, amide or sulphonate serving as ligands. The syntheses of the soluble macrocomplexes were achieved by employing methodology based on the reaction of H_2WO_4 with H_2O_2 and respective polymeric ligand in an aqueous medium under fairly mild condition at near neutral pH. Adopting a somewhat similar synthetic protocol, the insoluble compound **PANW** (**3.1**) supported on PAN was obtained by allowing the polymer to swell in a mixture containing the peroxotungsten species generated *in situ* from the reaction of H_2WO_4 with 30% H_2O_2 at pH *ca.* 5. The importance of pH for the successful synthesis of peroxo-metal compounds has been emphasized in the literature[1,10,11]. It has been known that a number of peroxotungsten species are formed in solution with slight variation of pH of the reaction medium. Also, mode and extent of co-ordination offered by water soluble polymers used as support in the present study are known to be pH sensitive[42-44]. For the synthesis of the title compounds, a pH of ca. 5 was found to be optimum for the formation

of peroxotungsten species and their anchoring to the pendant functional groups of the polymers. Maintenance of required time and temperature at ≤ 4 °C and limiting water to that contributed by 30% H_2O_2 and alkali hydroxide solution were the other essential components of the procedure. The insoluble microcrystalline product, **PANW (3.1)** was separated from the reaction mixture simply by filtration, whereas, the soluble compounds were obtained by solvent induced precipitation which is an effective way of isolating soluble polymeric compounds[22,23]. Acetone was used to facilitate the precipitation of the soluble compounds. The compounds remain stable in the solid state for several weeks stored dry in closed containers at < 30 °C.

3.3.1.2 Characterization

The elemental analysis data of the compounds **PAW (3.2)**, **PMAW (3.3)**, **PAmW (3.4)** and **PSW (3.5)** (Table 3.1) indicated the presence of two peroxide groups per metal centre in the compounds. In **PANW (3.1)**, on the other hand, W:peroxide ratio was found to be 1:1. The tungsten loading on the compounds based on elemental analysis and confirmed by EDX spectral analysis are presented in Table 3.1. The metal loading was found to vary from compound to compound with maximum being 100% anchoring of the ligand sites for the compound **PSW (3.5)**. The same was rather low for the insoluble compound **PANW (3.1)** possibly due to the heterogeneous reaction condition.

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

Scanning electron microscopy was used to investigate the particle size as well as morphological changes occurring on the surfaces of the polymers after loading of the

peroxotungstates to the polymer matrix. From the micrographs it was evident that metal ions are distributed across the surfaces of the polymeric peroxometal compounds (Fig. 3.1, 3.3 and 3.5). For the insoluble complex **3.1**, there is considerable enhancement of the average particle size after incorporation of the pW moieties into the polymer beads. The surface of each of the polymer anchored complexes **3.1-3.5** exhibited considerable roughening in contrast to the smooth and flat surfaces of the pristine polymers. Data obtained on the composition of the compounds from the energy dispersive X-ray spectroscopy, which provides *in situ* chemical analysis of the bulk, were consistent with the elemental analysis values (Table 3.1). EDX analysis was carried out focusing multiple regions over the surface of the polymer. The data presented in Table 3.1 is the averaging out of the data from these regions.

3.3.1.2.2 IR and electronic spectral studies

The significant features of IR spectrum of the polymer-anchored complexes **3.1-3.5** are summarized in Table 3.2 and presented in Fig. 3.7- Fig. 3.11. On the basis of available literature data on metal compounds with co-ordination environment comprising of ligands relevant to the present study, fairly reliable empirical assignments could be derived for the IR bands observed for the compounds. The presence of side-on bound peroxo ligand in the compounds was evident from the observance of the characteristic ν(O-O), ν_{asym}(W-O_2) and ν_{sym}(W-O_2) modes in the vicinity of ca. 860, ca. 610 and ca. 530 cm^{-1}, respectively[10,11]. The strong absorption at ca. 960 cm^{-1} have been assigned to ν(W=O) mode of terminally bonded W=O group[10,11].

Table 3.1 Analytical data of the polymer-bound peroxotungstate complexes **3.1 - 3.5**

Compound	% Found from elemental analysis (% obtained from EDX spectra)						Metal ion loading [a] (mmol g^{-1} of polymer)
	C	H	N	Na	W	O_2^{2-}	
PANW	63.41 (63.28)	2.86 ---	25.37 (26.09)	---	6.84 (6.97)	1.20	0.38
PAW	21.86 (22.61)	2.66 ----	---- ----	---- (12.47)	30.01 (30.06)	10.23 ----	1.63
PMAW	23.18 (23.50)	3.16 ----	----- -----	--- (15.40)	23.00 (23.14)	7.92 ----	1.25
PAmW	33.61 (34.28)	4.10 ---	13.61 (14.23)	--- ---	24.68 (23.44)	8.24 ----	1.28
PSW	5.51 (5.89)	0.89 ---	---- ----	---- (6.44)	43.52 (43.96)	15.00 ----	2.36

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

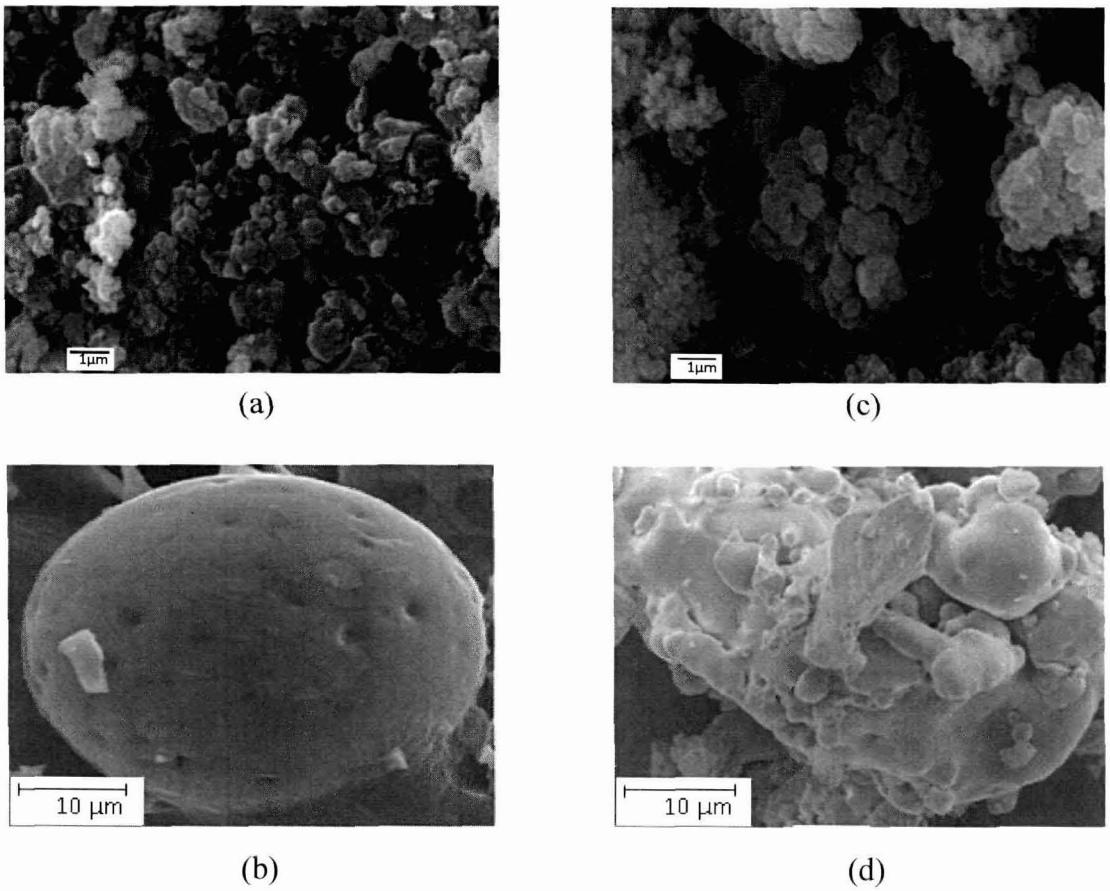


Fig. 3.1 Scanning electron micrographs of (a) PAN, (b) PA, (c) **PANW** and (d) **PAW**

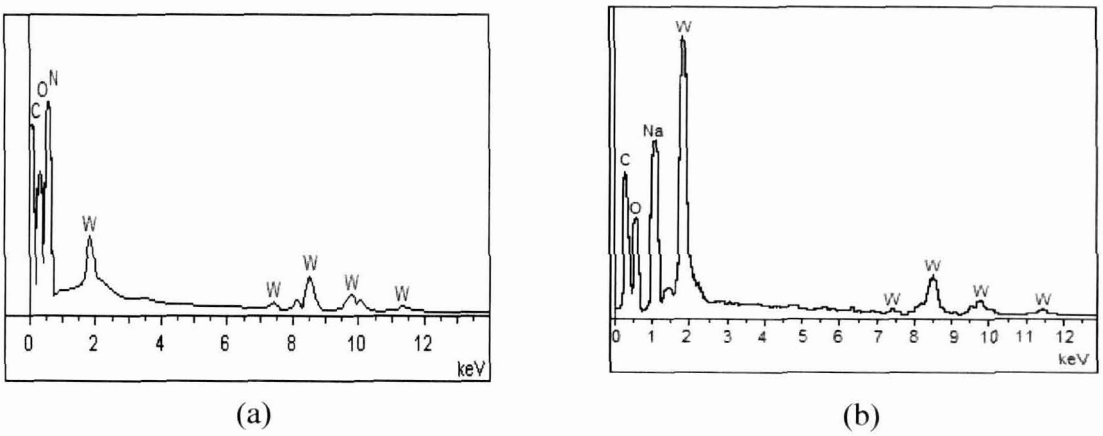
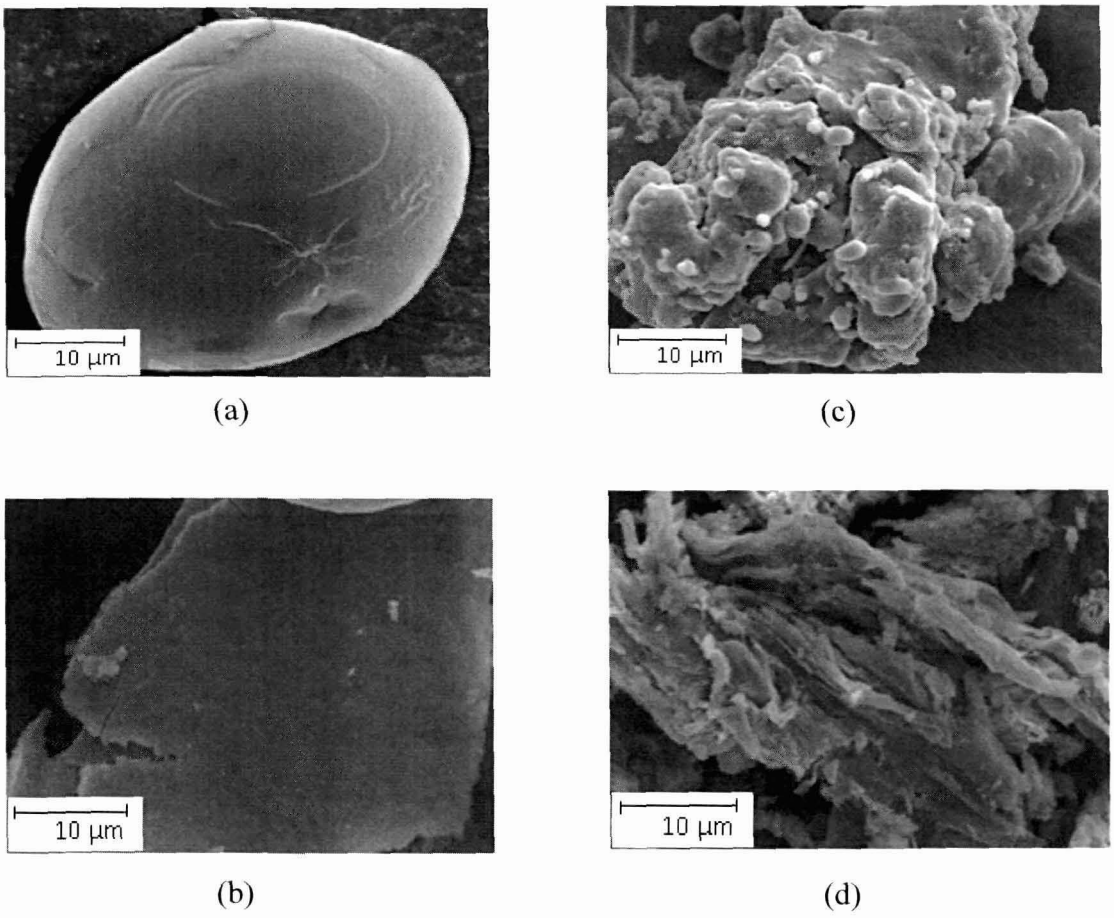


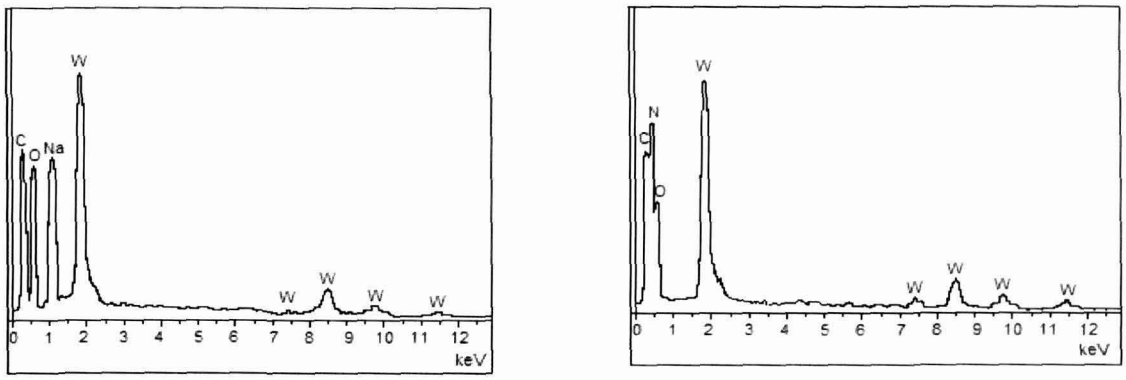
Fig. 3.2 EDX spectra of (a) **PANW** and (b) **PAW**



(a) (c)

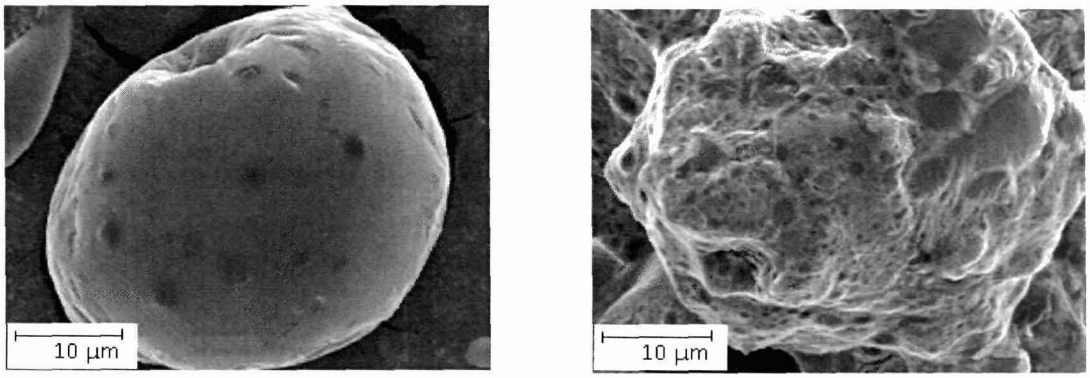
(b) (d)

Fig. 3.3 Scanning electron micrographs of (a) PMA, (b) PAm, (c) **PMAW** and (d) **PAmW**



(a) (b)

Fig. 3.4 EDX spectra of (a) **PMAW** and (b) **PAmW**



(a) (b)

Fig. 3.5 Scanning electron micrographs of (a) PS and (b) **PSW**

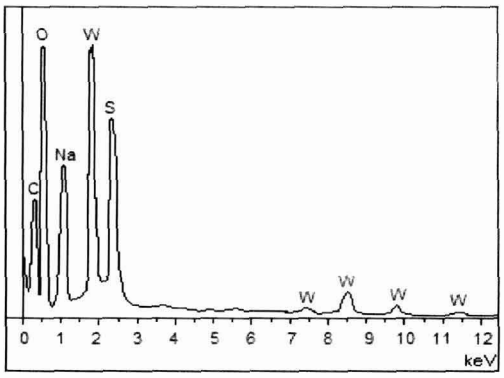


Fig. 3.6 EDX spectra of **PSW.**

In case of pristine polymer poly(acrylonitrile), a strong ν(C≡N) absorption observed at 2247 cm^{-1}.[45] In the spectrum of **PANW (3.1)**, in addition to the band at 2247 cm^{-1} for the free nitrile groups, a new medium intensity band was observed at 2366 cm^{-1}. This latter band is attributable to a shift of ν(C≡N) to a higher frequency, resulting from co-ordination of the W(VI) ion with the pendant nitrile group of the polymer. It has been documented earlier that in simple N-bonded nitrile complexes there is usually an increase in ν(C≡N) upon coordination[46].

It has been well established that ($\Delta\nu = \nu_{asym} - \nu_{sym}$) relationship with the carboxylato co-ordination, derived from thorough investigation on carboxylato complexes having known crystal structures[47,48], also holds for polycarboxylates[49,50] as well as for polyacrylates[51,52]. In the spectra of free polymers poly(acrylate) and poly(methacrylate) and the corresponding metal anchored compounds **PAW (3.2)** and **PMAW (3.3)** exhibited typical bands between 1710 and 1540 cm^{-1} owing to ν_{asym}(COO), and in the range of 1415 and 1406 cm^{-1} due to ν_{sym}(COO) mode. A close analogy was observed in the spectral patterns exhibited by the compounds **PAW (3.2)** and **PMAW (3.3)** as well as the pV and pMo compounds anchored to PMA, reported previously by us[29,30]. In the spectrum of pure polyacrylate, ν_{asym}(COO) and ν_{sym}(COO) modes are observed at 1565 and 1409 cm^{-1}, respectively ($\Delta\nu$= 156 cm^{-1}) whereas, in case of neat PMA the same appeared at 1540 and 1415 cm^{-1}, respectively ($\Delta\nu$ = 125 cm^{-1}). After incorporation of the pW species into these matrices the spectra of both the compounds showed a distinct shift of the ν_{asym}(COO) band to a higher frequency in the region 1650 to 1660 cm^{-1}, along with some broadening. The resulting $\Delta\nu$ (240 cm^{-1} for **PAW (3.2)** and 252 cm^{-1} for **PMAW (3.3)**) being much greater relative to the free PA or PMA gave clear indication of the presence of unidentately coordinated carboxylate groups in the compounds. The broadening of the

Table 3.2 Infrared Spectral Data for the pW Compounds **3.1-3.5**

PANW	**PAW**	**PMAW**	**PAmW**	**PSW**	Assignment
939(m)	947(vs)	969(vs)	961(vs)	954(vs)	ν(W=O)
858(m)	853(vs)	851(vs)	862(vs)	853(vs)	ν(O-O)
535(m)	530(m)	526(m)	508(m)	539(m)	ν_{sym}(W-O_2)
611(m)	606(m)	612(m)	602(m)	621(m)	ν_{asym}(W-O_2)
	1705(s) 1645(br, s) 1570(sh, m)	1705(s) 1660(br, s)			ν_{asym}(COO)
	1405(s)	1408(s)			ν_{sym}(COO)
				1220(vs) 1188(vs) 1046(s)	ν(S-O)
			1643(sh) 1659(br, s)		ν(C=O)
			1435(m)		ν(C-N)
2247(vs) 2366(m)					ν(C≡N)

vs, very strong; br, broad; s, strong; sh, shoulder; m, medium

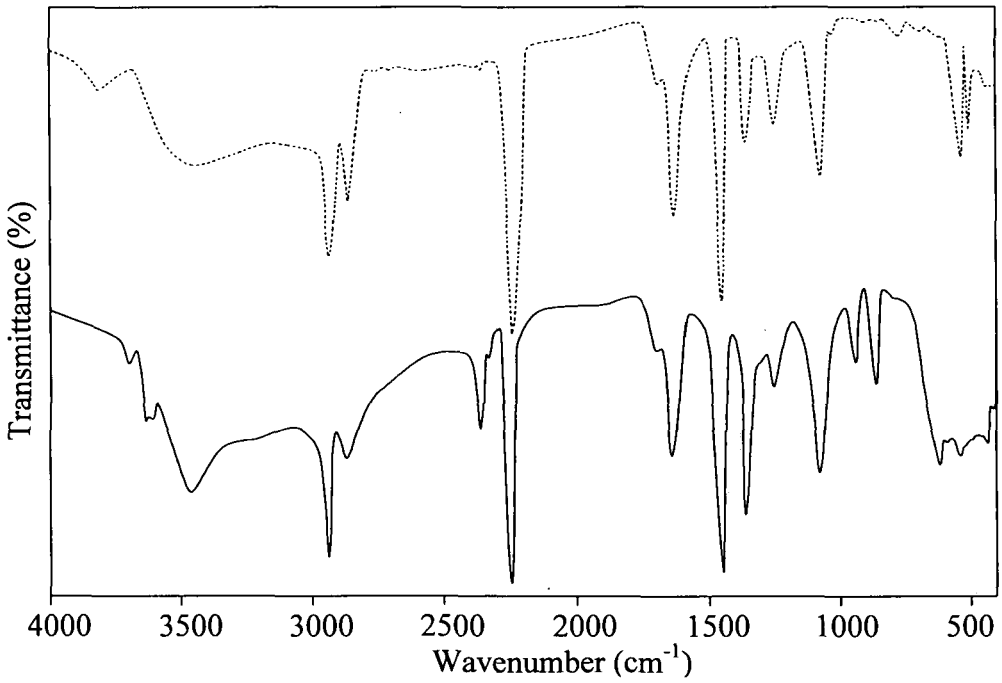


Fig. 3.7 IR spectra of **PANW (3.1)** (solid line) and PAN (broken line)

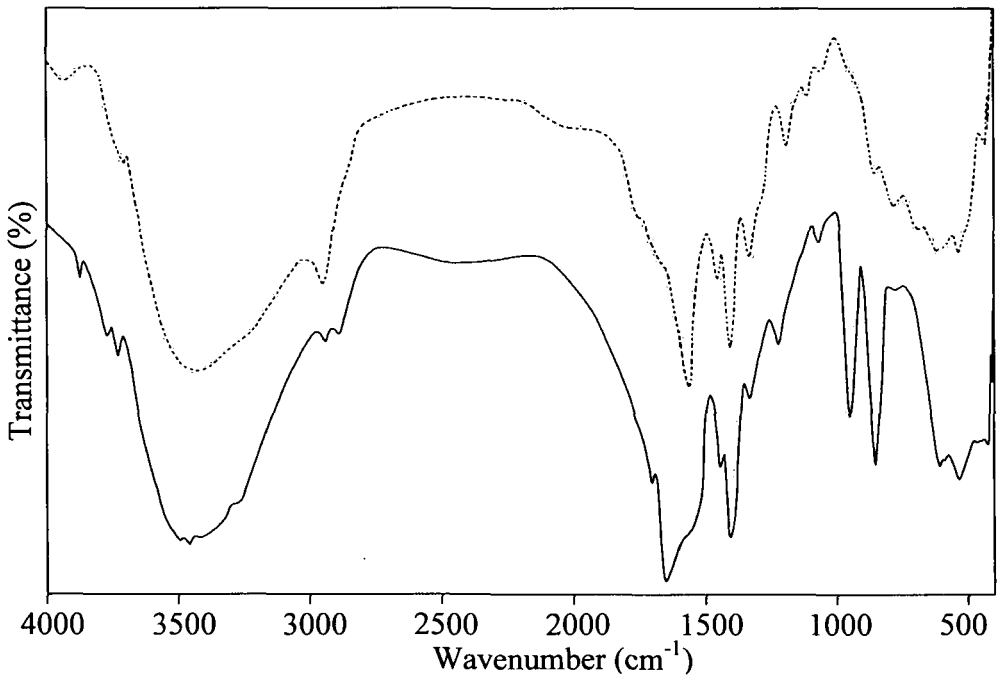


Fig. 3.8 IR spectra of **PAW (3.2)** (solid line) and PA (broken line)

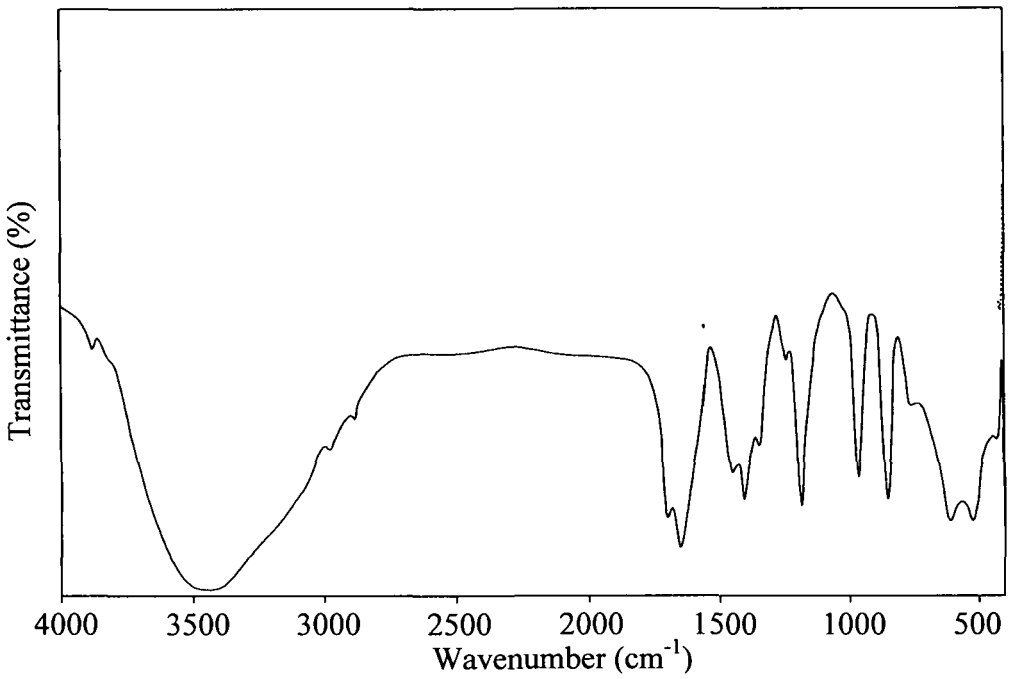


Fig. 3.9 IR spectra of **PMAW (3.3)** (solid line) and PMA (broken line)

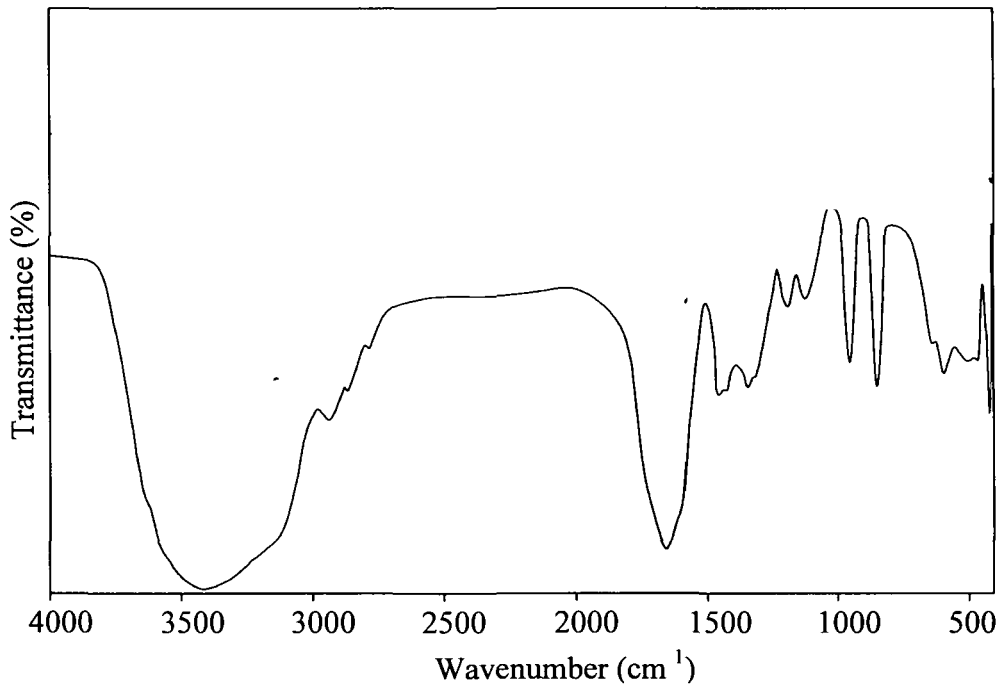


Fig. 3.10 IR spectra of **PAmW (3.4)** (solid line) and PAm (broken line)

band in the spectra of the compounds and a shoulder identified at 1570 cm^{-1} in the spectrum of **PAW (3.2)** are likely to be due to the presence of uncoordinated carboxylates in the compound. In each of the compounds **PAW (3.2)** and **PMAW (3.3)** the presence of free –COOH groups was evident from the additional band appearing in the vicinity of ca. 1705 cm^{-1}. Metal oxygen vibration in the spectra of these compounds were identified as weak bands in the far IR region between 500 and 400 cm^{-1}.

In the spectrum of the pristine poly (acrylamide), the ν(C=O) appeared as intense band at 1643 cm^{-1}. The amide groups of poly (acrylamide) have two potential alternative metal binding sites viz. amide nitrogen or the carbonyl oxygen[53-58]. Co-ordination through lone pair of nitrogen is known to cause an increase in ν(C=O) (amide-I) band frequency whereas bonding via carbonyl oxygen shifts the carbonyl absorption to lower value[53-55]. In the spectrum of **PAmW (3.4)** with complexed amide groups, in addition to the band at 1643 cm^{-1}, a new characteristic band appeared in the carbonyl region at 1659 cm^{-1}. This later band is attributable to a shift of the amide-I absorption to a higher frequency resulting from the co-ordination of the W(VI) ion with N (amide) atom. N-H stretching could not be assigned with certainty as it occurred in the O-H frequency region. The ν(C-N) was identified as a medium intensity band at 1447 cm^{-1}.

In the spectrum of the neat polymer, poly (vinyl sulfonate), the bands corresponding to S-O stretching of the pendant sulfonate group occur at 1210 and 1127 cm^{-1}, respectively[53]. The band at ca. 1040 cm^{-1} is due to the symmetric stretching vibration of sulfonate anion[58]. The spectrum of the compound **PSW (3.5)** shows a distinct splitting pattern displaying bands at 1220 and 1188 cm^{-1} in addition to antisymmetric vibration of S-O at 1128 cm^{-1}, that we attribute to complexed sulfonate group[53,58-60].

Presence of lattice water in the complexes **3.2-3.5** was evident from the appearance of strong and broad ν(OH) absorptions displayed at 3500-3400 cm^{-1}.

Electronic spectrum of the compounds **3.2-3.5,** recorded in aqueous solution exhibited a weak intensity broad band at 230-250 nm which was attributable to peroxo to metal (LMCT) transition. The band was observed in the region characteristic of a diperoxotungstate species[1,61]. For the compound **PANW (3.1)**, a band has been expected in the range of 320-330 nm, due to LMCT transitions originating from co-ordinated peroxide of monoperoxo derivatives of tungsten[62,63]. However, no such peak was observed in the spectrum of the compound probably due to the low metal loading on the polymer matrix.

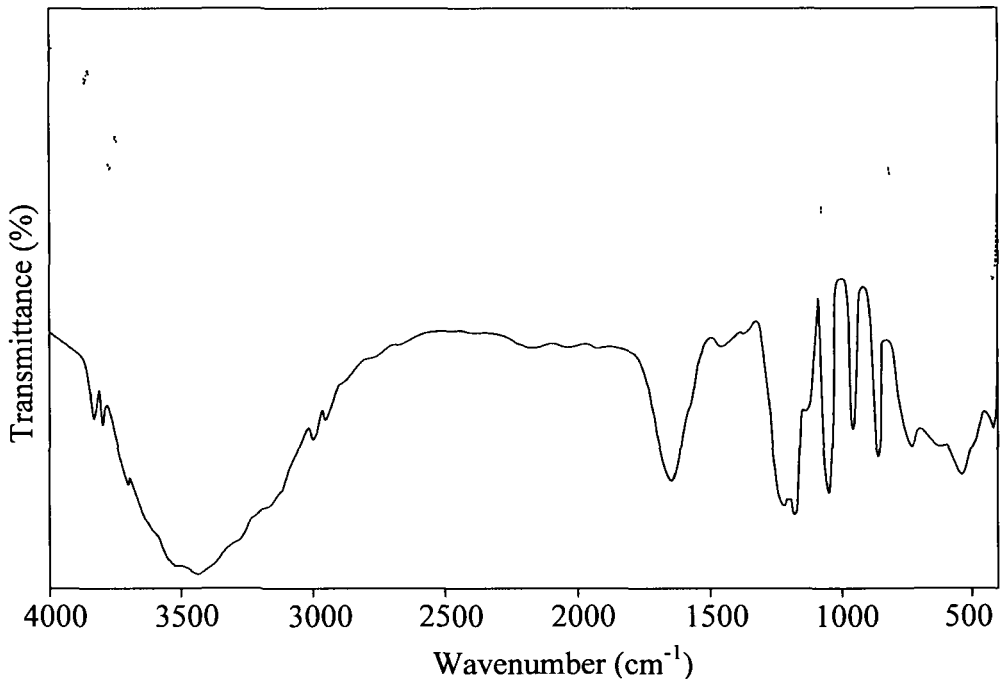


Fig. 3.11 IR spectra of **PSW (3.5)** (solid line) and PS (broken line)

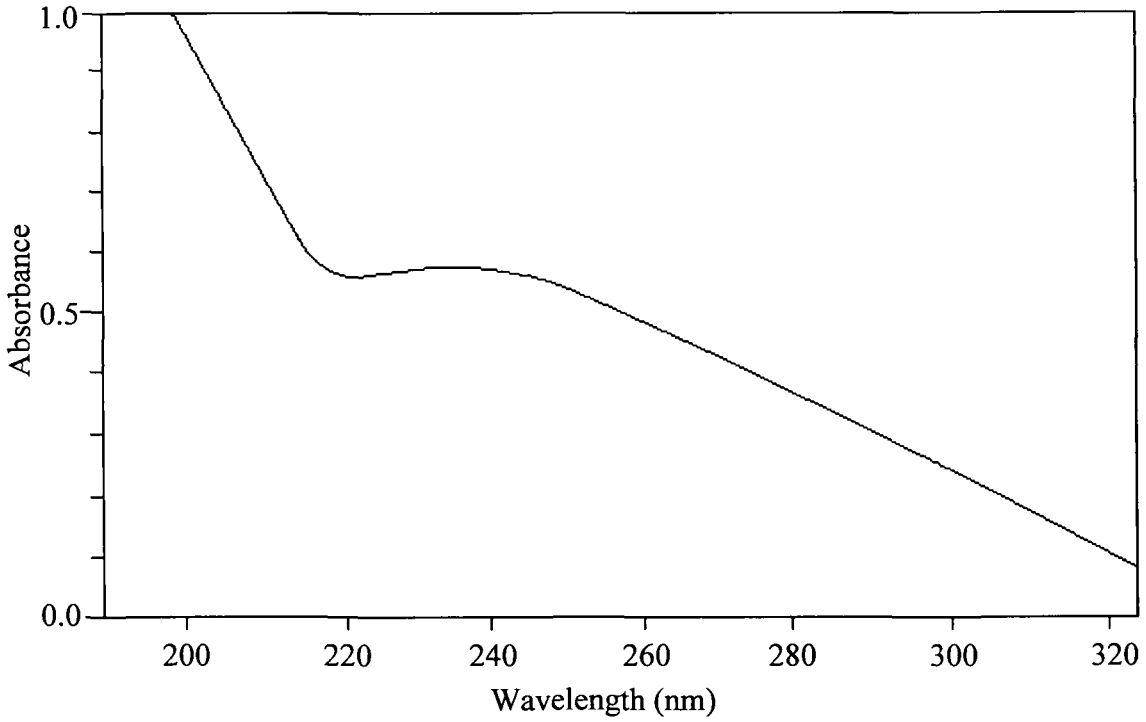


Fig. 3.12 UV spectra of **PAW (3.1)**

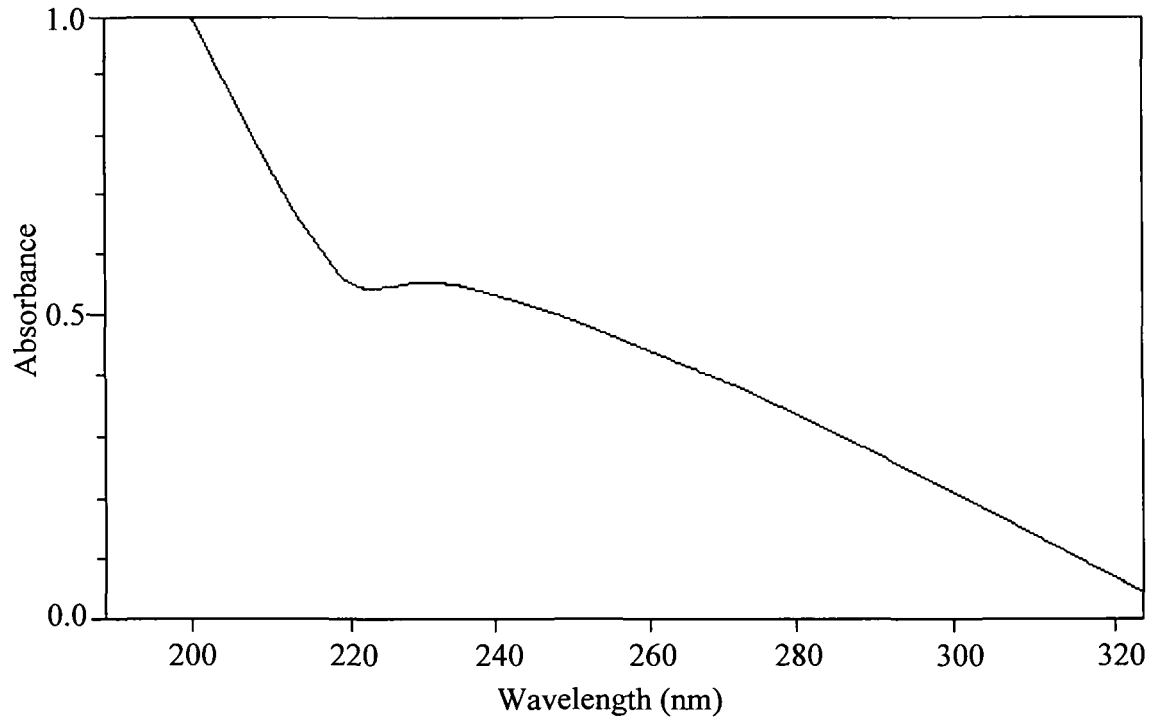


Fig. 3.13 UV spectra of **PMAW (3.3)**

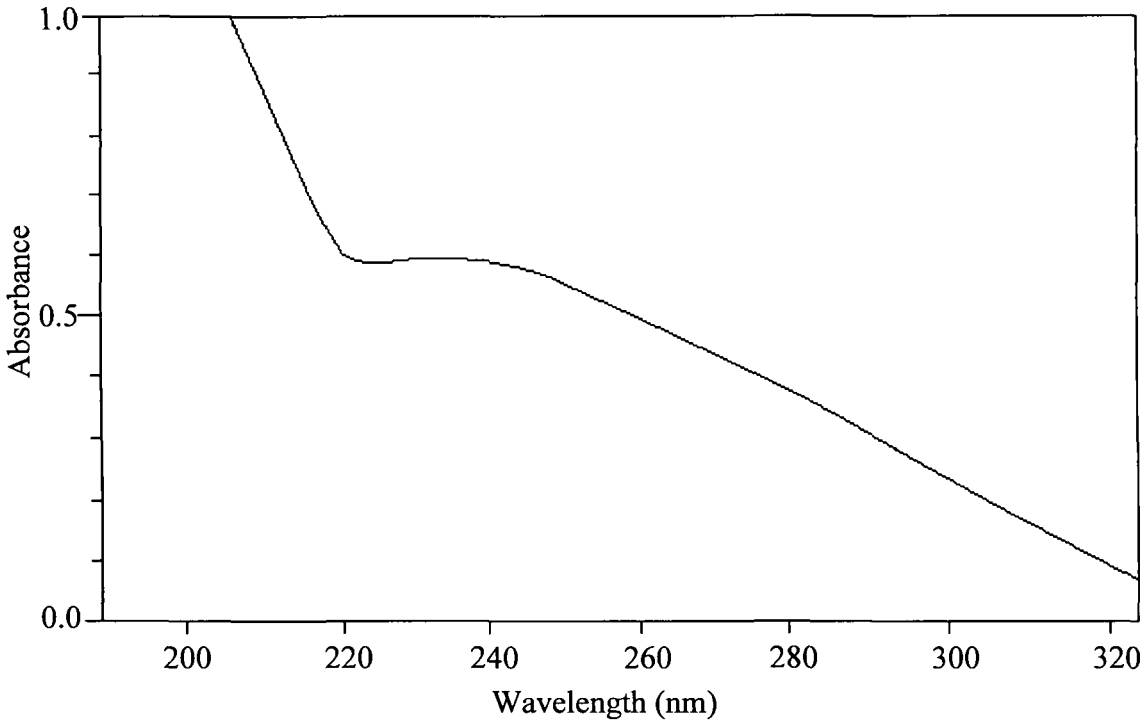


Fig. 3.14 UV spectra of **PAmW (3.4)**

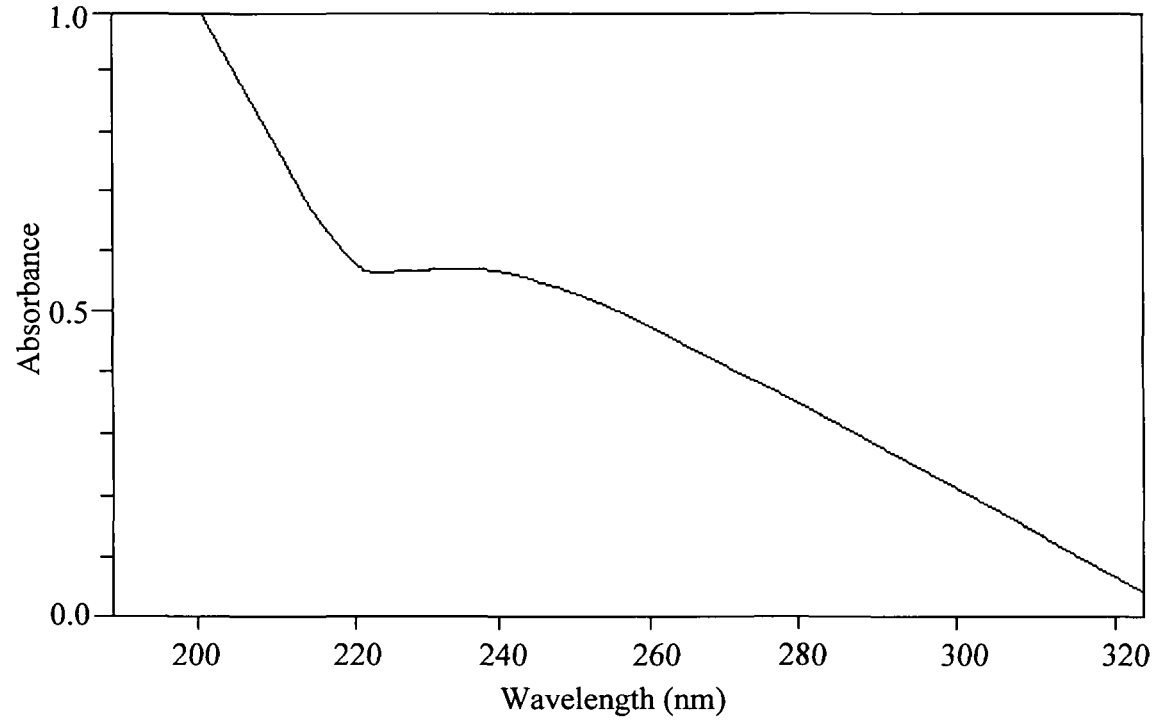


Fig. 3.15 UV spectra of **PSW (3.5)**

3.3.1.2.3 ^{13}C NMR Studies

The study of co-ordination induced ^{13}C NMR chemical shift has been recognized as an important tool in understanding the mode of coordination of the co-ligands in peroxo metal compounds[11,29,64-68]. Crucial information regarding bonding pattern of the macromolecular ligands to the metal centres in the compounds and their stability in solution was provided by ^{13}C NMR data. The assignments of major peaks were made on the basis of available literature data[11,64-72]. The ^{13}C NMR spectra and data for the polymer-anchored complexes **3.1-3.5** and respective pure polymers are presented in Fig. 3.16 – Fig. 3.19 and Table 3.3, respectively. The ^{13}C NMR spectra of the pure poly(acrylonitrile) displays resonance due to pendant nitrile group at 120.21 ppm and the characteristic signals corresponding to chain carbon atoms at 33.35 (for CH_2) and 27.97 (for CH) ppm[73]. The spectrum of **PANW (3.1)** exhibited a new signal at 128.48 ppm attributable to tungsten bound nitrile group in addition to the signal at δ 120.15 ppm owing to free nitrile group of the polymer. It has been documented that on co-ordination to a metal, the nitrile carbon resonance undergoes a downfield shift[74]. The spectrum of **PANW (3.1)** thus evidenced for the presence of both coordinated as well as free nitrile groups. The substantial downfield shift, $\Delta\delta$ ($\delta_{complexed\ nitrile}$ - $\delta_{free\ nitrile}$) $\approx$ 8 ppm in the metal anchored compound relative to the free nitrile peak of the pristine polymer suggests strong metal-ligand interaction.

In the spectra of the pure polymers poly(acrylate) and poly(methacrylate), resonances due to the carboxylate carbon atoms centered at 184 and 187 ppm, respectively, in addition to the characteristic signals corresponding to chain carbon atoms[69-72]. Two closely spaced peaks observed in this region is likely to be due to the

Table 3.3 ^{13}C NMR Chemical Shift for Polymer-Anchored peroxotungstate Compounds **3.1-3.5** and Base Polymers

Compound	Chemical Shift (ppm)				
	Carboxylate/amide/nitrile carbon		CH_2	CH	CH_3
	Free	Complexed			
PAN	120.21	128.48	33.35	27.97	
PANW	120.15		33.34	27.97	
PA	184.50	216.03	36.10	45.52	
PAW	185.15		36.72	46.12	
PMA	187.41	215.22	17.36		56.54
PMAW	186.64		17.12		55.93
PAm	179.48	200.26	34.88	41.66	
PAmW	179.51		34.89	41.66	
PS			30.97	54.51	
PSW			30.92	54.54	

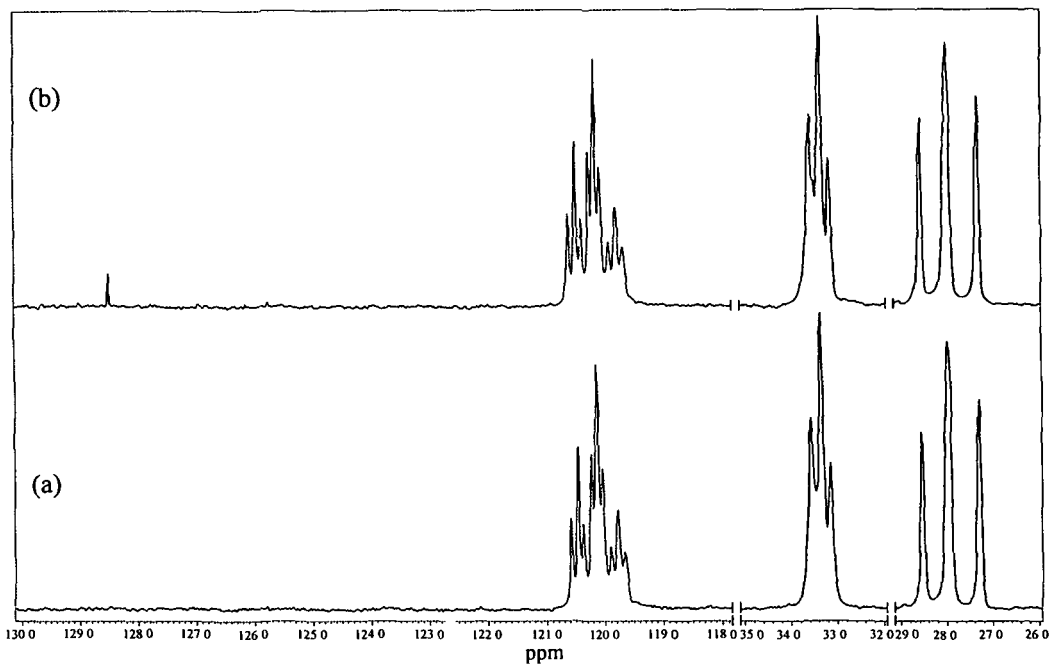


Fig. 3.16 ^{13}C NMR spectra of (a) PAN and (b) **PANW**

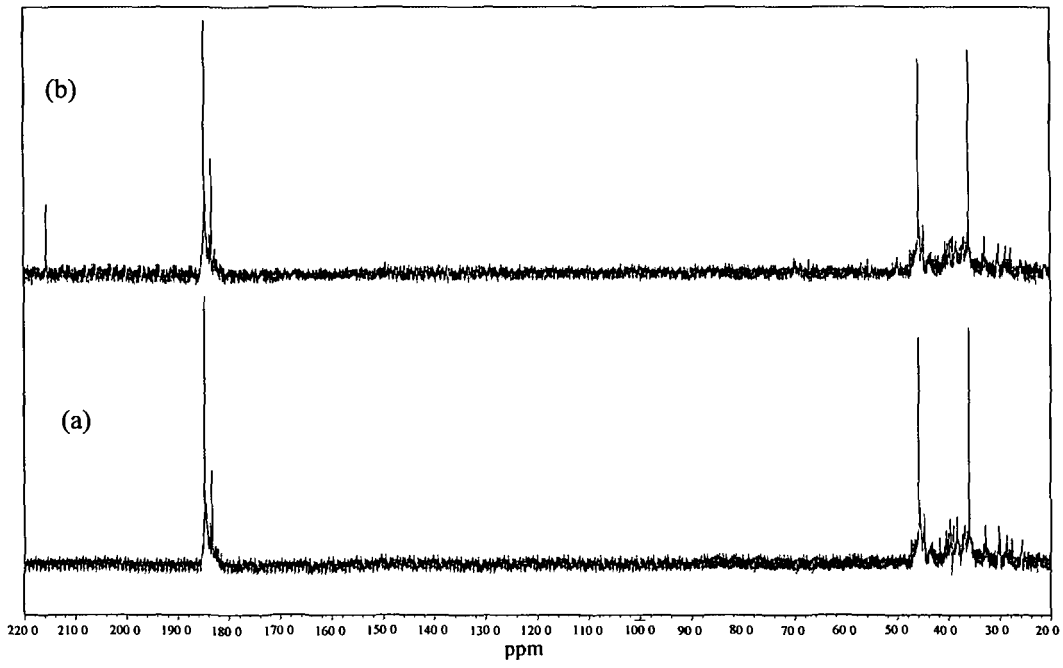


Fig. 3.17 ^{13}C NMR spectra of (a) PA and (b) **PAW**

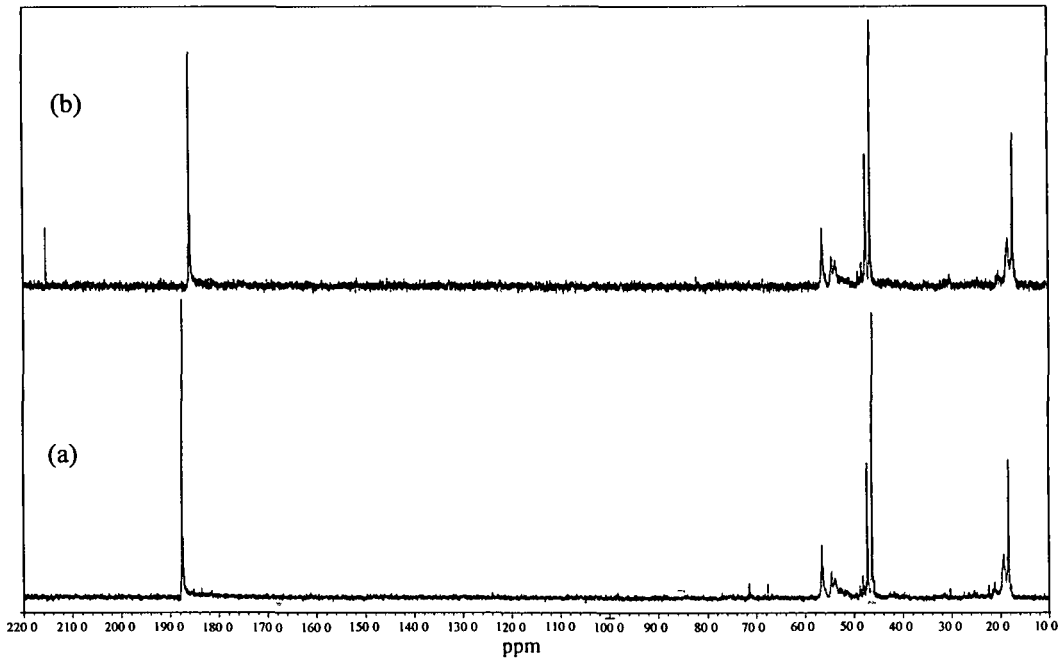


Fig. 3.18 ^{13}C NMR spectra of (a) PMA and (b) **PMAW**

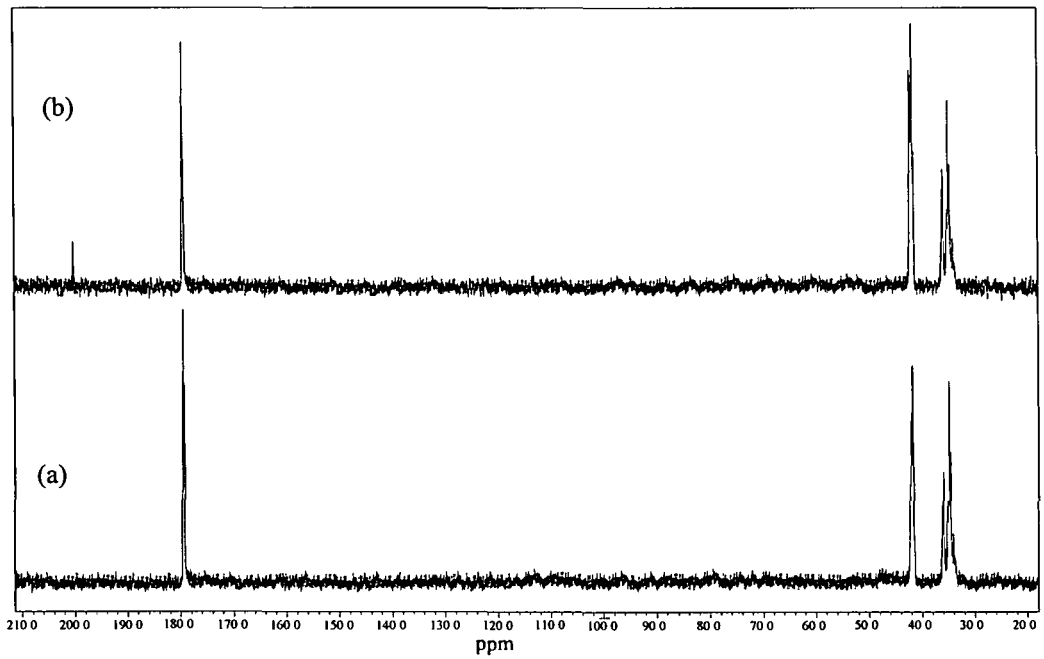


Fig. 3.19 ^{13}C NMR spectra of (a) PAm and (b) **PAmW**

presence of free carboxylate as well as –COOH groups of the polymers in solution. The spectra of the pW anchored polymeric compounds **PAW (3.2)** and **PMAW (3.3)** showed the presence of complexed as well as free carboxylate groups by displaying a new peak at lower field of ca. 215 ppm, in addition to the characteristic carboxylate resonance corresponding to the free carboxylate groups as observed in the pure polymers (Table 3.3). Strong metal ligand interaction was indicated by the large downfield shift, $\Delta\delta$ ($\delta_{complex}$ - $\delta_{free\ carboxylate}$) = 30.88 ppm in case of PA bound compound and 28.58 ppm in the metal anchored PMA compound relative to the free carboxylate peak of the pristine polymer.

Detailed information are available in the literature on the ^{13}C NMR spectral analysis of poly (acrylamide) under varying pH conditions[75]. The spectrum of pW incorporated **PAmW (3.4)** displayed, in addition to the resonance at 179.51 ppm corresponding to the free amide groups as observed in the pure poly (acrylamide) (Table 3.3), a new peak at 200.26 ppm. This resonance is attributable to carbon atom of the tungsten co-ordinated amide group. In the spectrum of the compound **PSW (3.5)**, no significant change was noted in the positions of the peaks corresponding to CH and CH_2 groups of the polymer chain compared to the pure polymer, poly (vinyl sulfonate). This may not be unusual considering that W atoms are bound to the polymer through the sulfonate groups and hence are well separated from the chain carbon atoms of the polymer support.

The resonance due to complexed nitrile or carboxylate or amide occurring as a singlet in the spectra of polymer anchored compounds, **PANW (3.1)**, **PAW (3.2)**, **PMAW (3.3)** and **PAmW (3.4)**, evidenced for a single carbon environment, obviously resulting from a single mode of metal-ligand co-ordination, in agreement with the proposed structure. The findings from the ^{13}C NMR spectral analysis of the compounds are consistent with retention of their solid state structures in solution.

3.3.1.2.4 Thermal analysis

The TG-DTG plots for the compounds **3.1-3.5** and the corresponding thermogravimetric analysis data are presented in Fig. 3.20 - Fig. 3.24 and Table 3.4, respectively, show that the compounds gradually undergo multistage decomposition on heating up to a final temperature of 750 °C. It is notable that the complexes, unlike some monomeric peroxometal compounds[12], do not explode on heating.

The first stage of decomposition occurring for the compounds **3.2-3.5** in the temperature range of ca. 74-101 °C, correspond to liberation of lattice water from the complexes with corresponding weight loss of 1.10% (**PAW**), 0.97% (**PMAW**), 1.01% (**PAmW**) and 1.02% (**PSW**). No such decomposition stage was observed in the thermogram of **PANW,** which confirms the absence of water of crystallization in this compound. The second decomposition stage in the temperature range of 112-210 °C, attributable to complete loss of co-ordinated peroxo groups from the complexes, was observed as a common feature in the thermograms of all the title compounds. Absence of peroxide in the decomposition product, isolated at this stage, was confirmed from the IR spectral analysis.

The next decomposition step in **PANW** (**3.1**) occurred between 305-360 °C which appeared as a single peak in DTG. The degradation further continued up to 750 °C. IR spectrum of the residue obtained at 360 °C showed bands at 1578 cm^{-1} and 1625 cm^{-1} characteristic of ν(C=C) and ν(C=N) stretching in addition to ν(W=O) at 957 cm^{-1}. The results are in general agreement with previous work where it was found that the degradation of PAN below 400 °C is accompanied by elimination of HCN, NH_3 and H_2O

Table 3.4 Thermogravimetric data of peroxotungsten complexes **3.1-3.5**

Compound	Temperature range(^{O}C)	Observed weight loss(%)	Final residue(%)
PANW	115-141	1.21	59.82
	305-360	12.83	
	360-750	26.14	
PAW	76-96	1.10	63.53
	119-172	10.46	
	324-551	24.91	
PMAW	74-101	0.97	65.86
	112-187	8.05	
	301-543	25.12	
PAmW	76-101	1.01	
	115-190	8.21	43.33
	200-330	14.24	
	330-451	33.21	
PSW	76-101	1.02	78.55
	116-210	15.24	
	378-440	1.88	
	440-570	3.31	

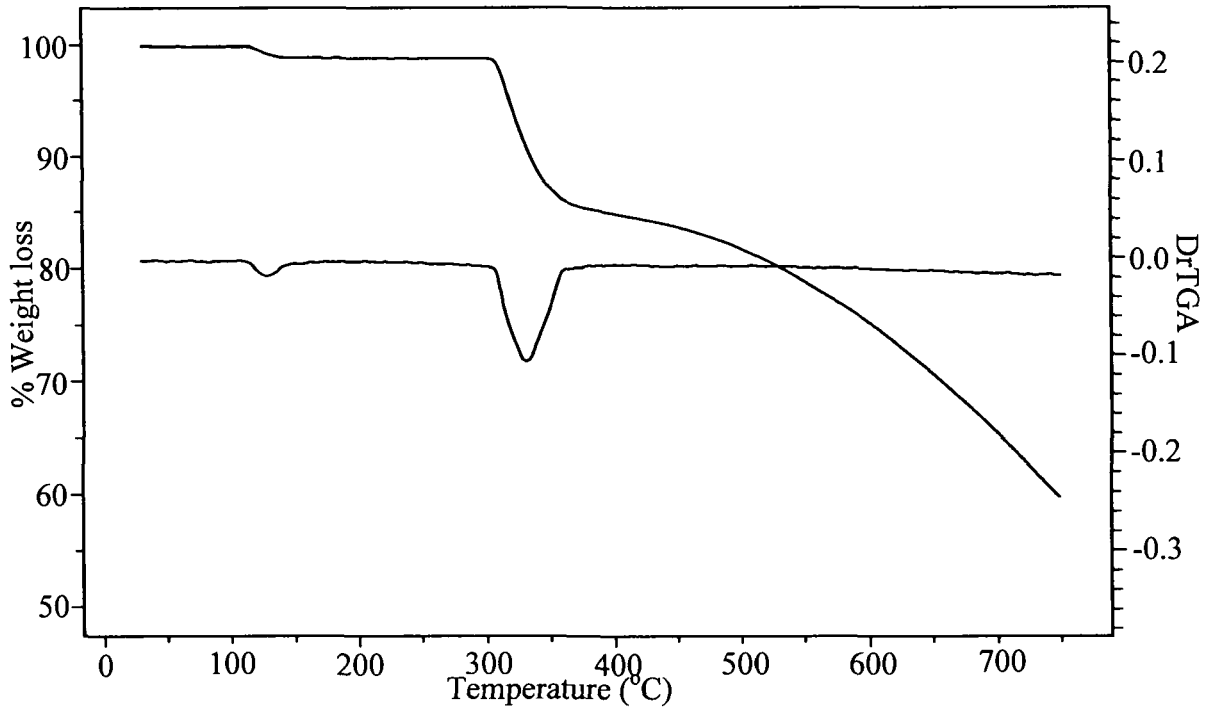


Fig. 3.20 TG-DTG of **PANW**

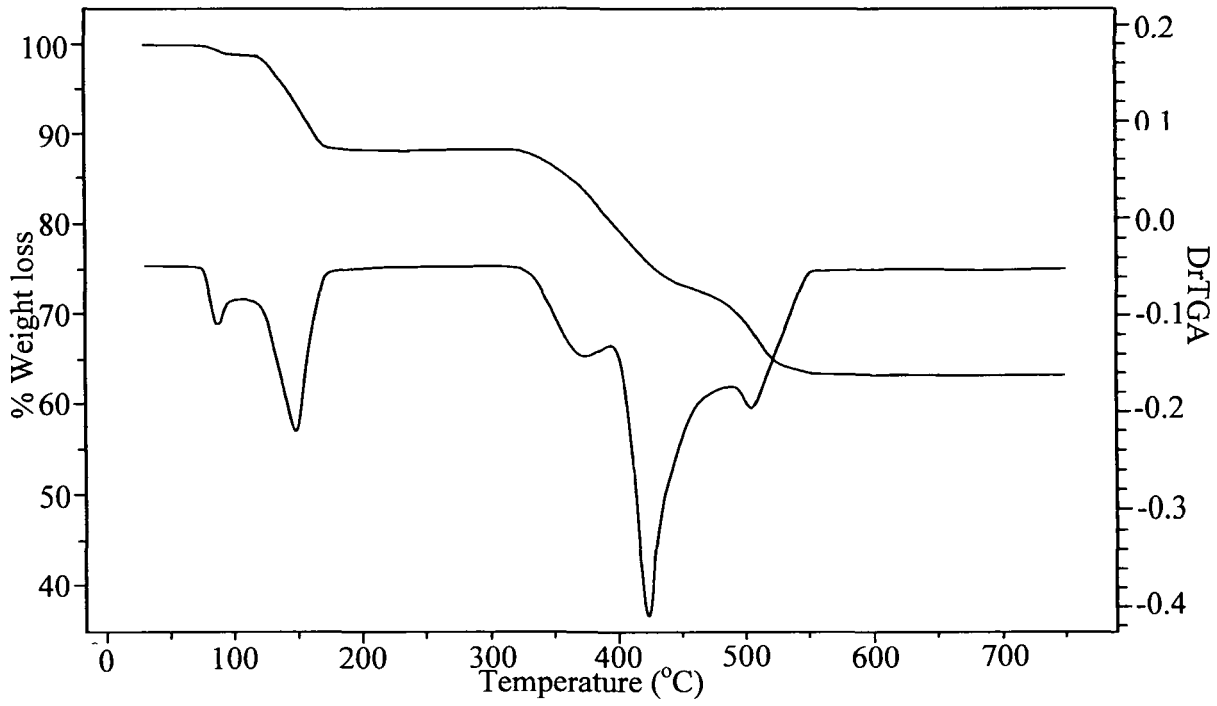


Fig. 3.21 TG-DTG of **PAW**

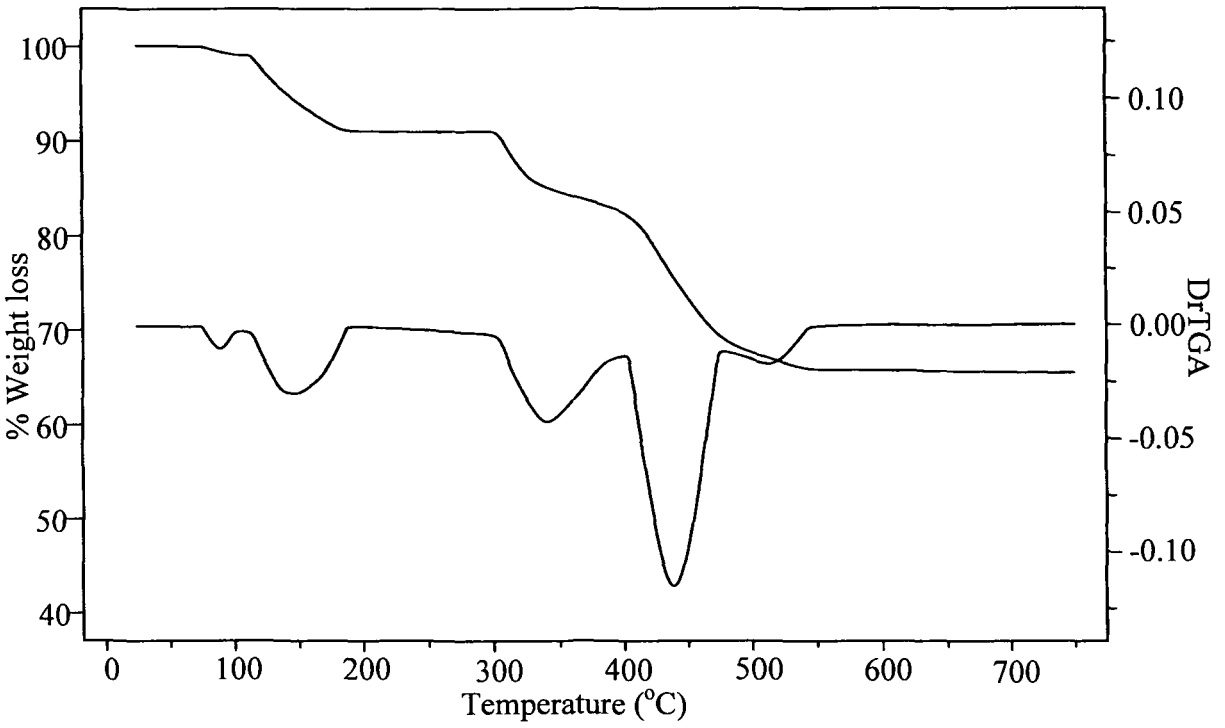


Fig. 3.22 TG-DTG of **PMAW**

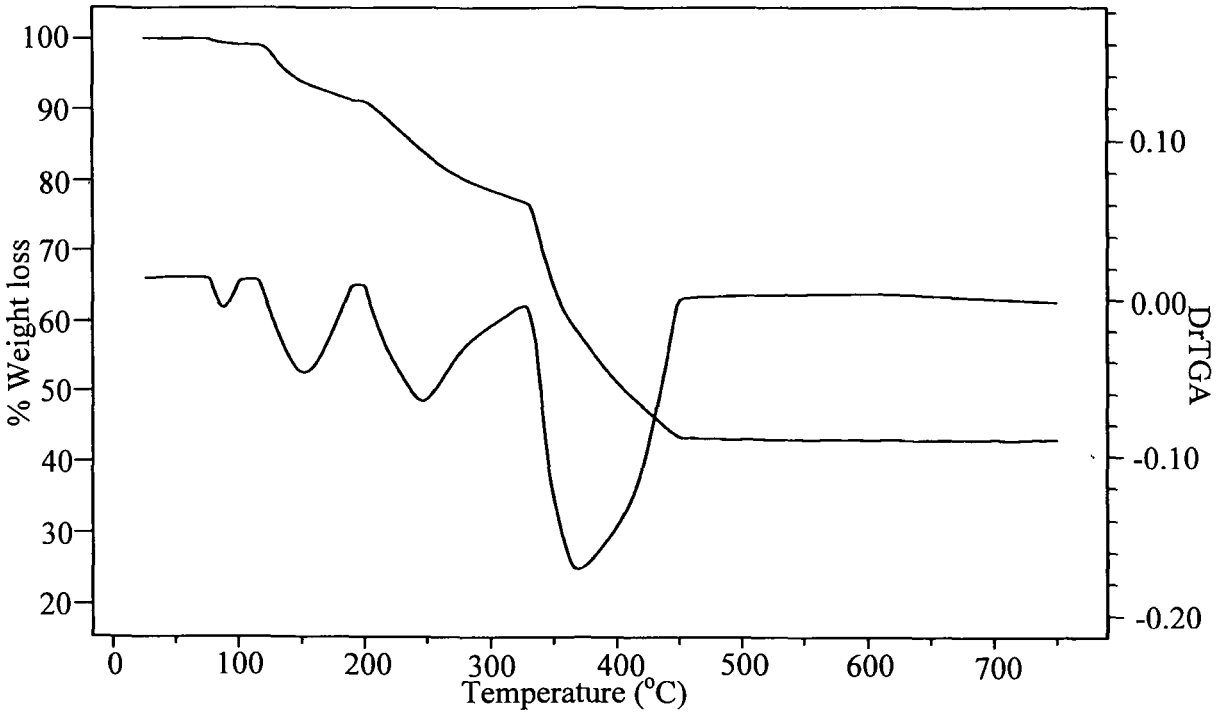


Fig. 3.23 TG-DTG of **PAmW**

and concomitant intramolecular polymerisation of nitrile groups to form conjugated polyamine $(-C=N)_n$ and that the loss of nitrogen commences at 750 °C[76].

The loss of peroxide is followed by three stage decomposition occurring in the temperature range of 324-551°C for **PAW (3.2)** and 301-543 °C for **PMAW (3.3)**, respectively which may be ascribed to decarboxylation involving free as well as coordinated carboxylate functionals accompanied by rupturing of the polymers. Further evidence in support of the decarboxylation of polymers was provided by IR spectra recorded after heating the compounds separately up to the final decomposition temperature which showed disappearance of the peaks originating from carboxylate stretching of the spectra of the original compounds.

The compound **PAmW (3.4)** with poly (acrylamide) as macroligand, after the loss of coordinated peroxo groups, undergo two weight loss processes in the temperature range of ca. 200-451 °C, attributable to breakdown of the polymer ligand. On the basis of the thermal decomposition patterns reported for some poly(acrylamides)[77], the first stage of decomposition in the temperature range 200–330 °C is ascribed to the release of water, ammonia and small amount of carbon dioxide, from the side-chain amide groups with the polymer chains remaining intact[77]. In the second stage of decomposition (330–451 °C) main chain breakdown occurs accompanied by majority of weight loss (33.21%).

In case of **PSW (3.5)**, a two stage decomposition was observed to follow the decomposition corresponding to the loss of peroxide. By analogy with the data available pertaining to the thermal degradation of poly(vinyl sulfonate) sodium salt, these decompositions in the range of 378-440 °C and the last one commencing at 440 °C and ending at 570 °C, are attributed to loss of sulfonate group and rupturing of the polymer accompanied by evolution of ethylene, water and SO_2 and CS_2[78].

The total weight loss which occurred during the course of the overall decomposition process viz. loss of lattice water, coordinated peroxide and polymeric functional, on heating the compound up to a final temperature of 750 °C was recorded to be 59.82% for **PANW (3.1)**, 36.47% for **PAW (3.2)**, 34.14% for **PMAW (3.3)**, 56.67% for **PAmW (3.4)** and 21.45% for **PSW (3.5)**. The IR spectra of the residue remaining at this stage showed the presence of oxotungstate species. Thermogravimetric analysis data of the compounds thus provided further evidence in support of their composition and formula assigned.

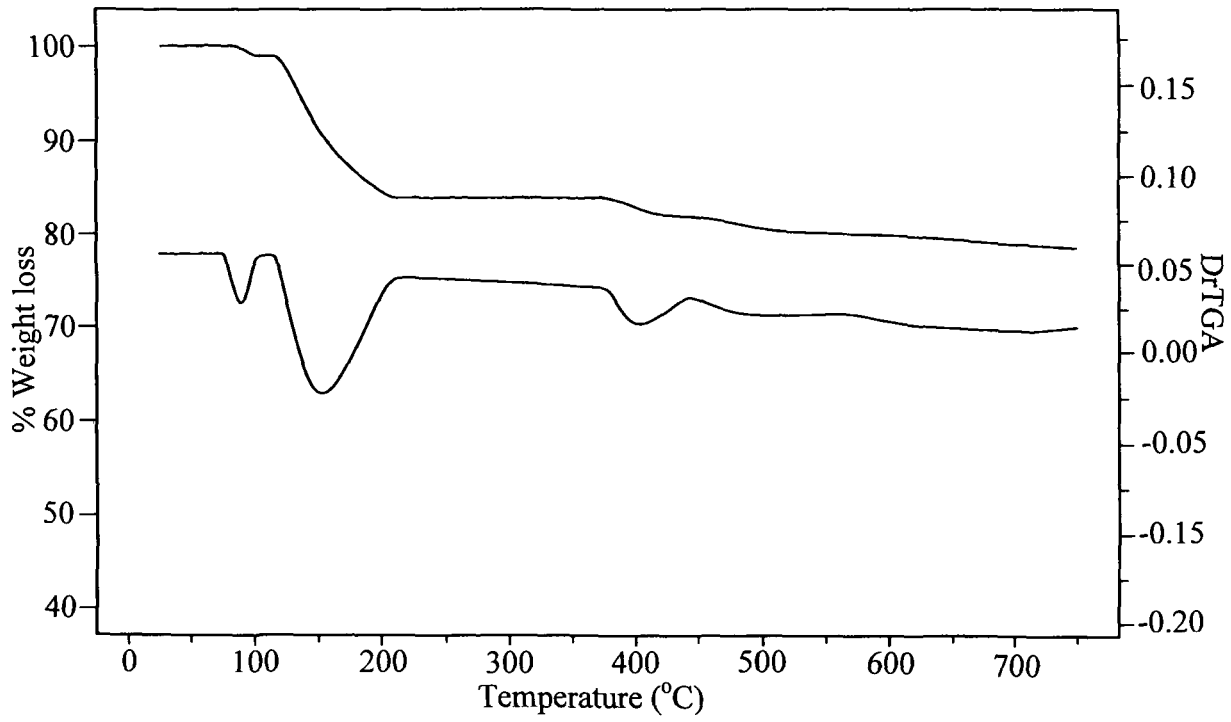


Fig. 3.24 TG-DTG of **PSW**

Based on the above data, the proposed structure of the insoluble polymer anchored pW complex **PANW (3.1)**, that includes tungsten(VI) atom with a side-on bound peroxo and terminal W=O groups, bonded to the polymer matrix via the N atom of the pendant nitrile groups, is shown schematically in Fig 3.25. Simultaneous bond formation of W(VI) to the two neighbouring nitrile groups of the polymer chain appears to complete the hexaco-ordination around each tungsten atom. For the compounds **PAW (3.2)** and **PMAW (3.3)**, a structure of the complex species, incorporating unidentate co-ordination via O-atoms of carboxylate groups of the polymer to a pW unit has been envisaged (Fig. 3.26 and Fig. 3.27). In the compounds **PAmW (3.4)** and **PSW (3.5)**, the pW species are similarly attached to the macromolecular ligand through N-atom of amide group or O-atom of sulfonate groups, respectively in unidentate fashion (Fig. 3.28 and Fig. 3.29). It is notable that in **PANW (3.1)**, the pW groups are attached in its dioxomonoperoxo form in contrast to the soluble complexes viz. **PAW (3.2), PMAW (3.3), PAmW (3.4)** and **PSW (3.5)**, where they occur as oxodiperoxotungsten(VI) species.

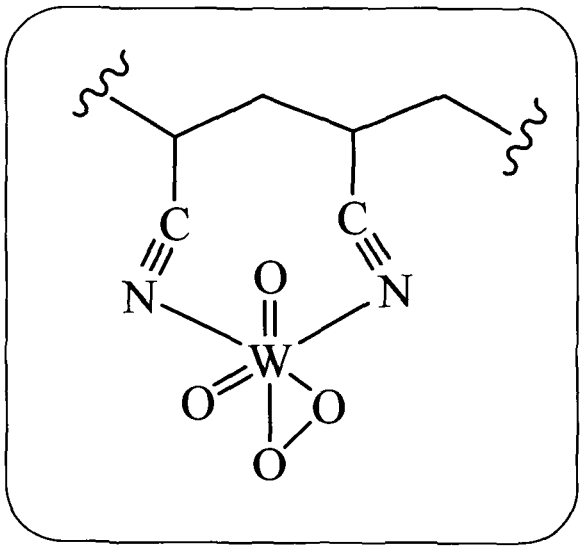


Fig. 3.25 Proposed structure of **PANW (3.1)**

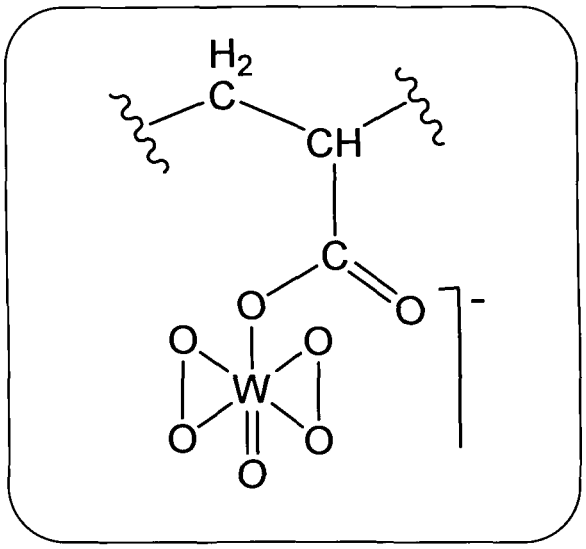


Fig. 3.26 Proposed structure of **PAW (3.2)**

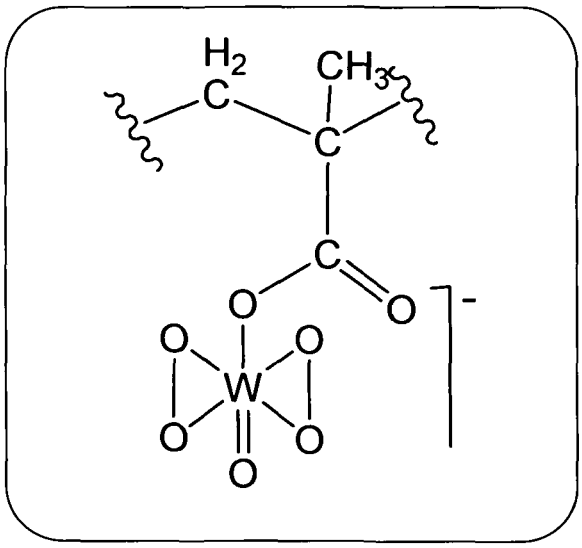


Fig. 3.27 Proposed structure of **PMAW (3.3)**

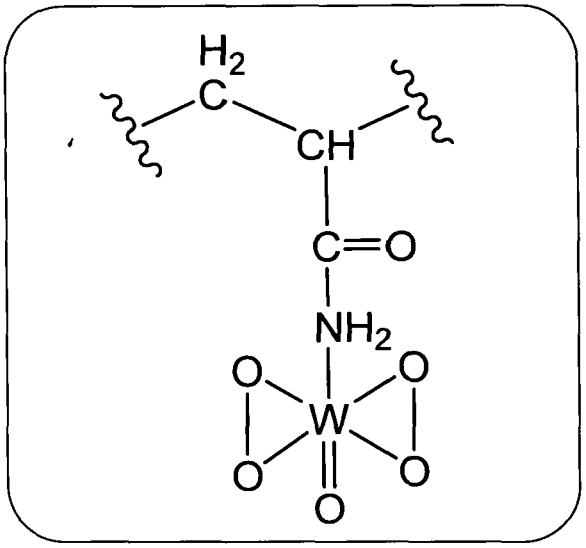


Fig. 3.28 Proposed structure of **PAmW (3.4)**

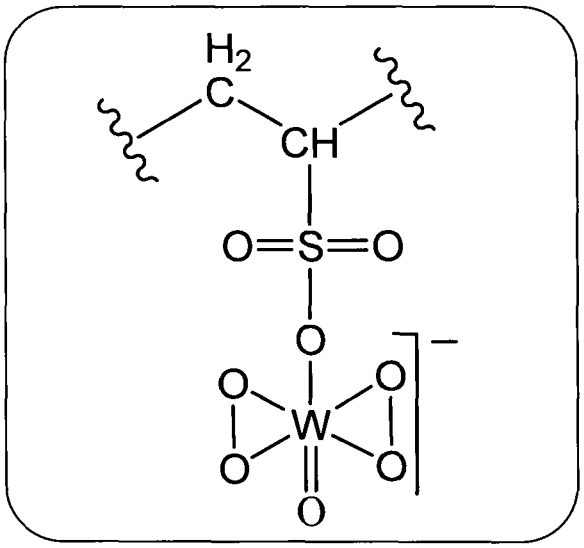


Fig. 3.29 Proposed structure of **PSW (3.5)**

3.3.2 Stability of the soluble complexes PAW (3.2), PMAW (3.3), PAmW (3.4) and PSW (3.5) in aqueous solution

One of the most important criteria required to be met by water soluble metal complexes to be useful as therapeutic or bio-relevant agent is the hydrolytic stability[79]. We have therefore considered it imperative to ascertain the stability of the compounds in solution not only at pH ca. 5, the natural pH attained by the solution on dissolving the compounds, but also under varying pH conditions ranging from 1.2 to 8.0. The stability of compounds with respect to the loss of peroxide in solution have been examined by estimating their peroxide content and absorbance at 230–250 nm region in the electronic spectra, at specified time intervals, for any possible change. The studies revealed that the peroxide content and position and intensity of their electronic spectral bands remained unaltered even after a period of over 12 h (Fig. 3.30). Moreover, the ^{13}C NMR spectra of the compounds recorded after 24 h of solution preparation showed no change in the spectral patterns suggesting that metal co-ordination to amide or carboxylate groups remained unchanged in the compounds in solution (Fig. 3.31). Thus all the evidences gathered, clearly attest to the stability of the compounds in solution not only at higher pH, but also under acidic conditions.

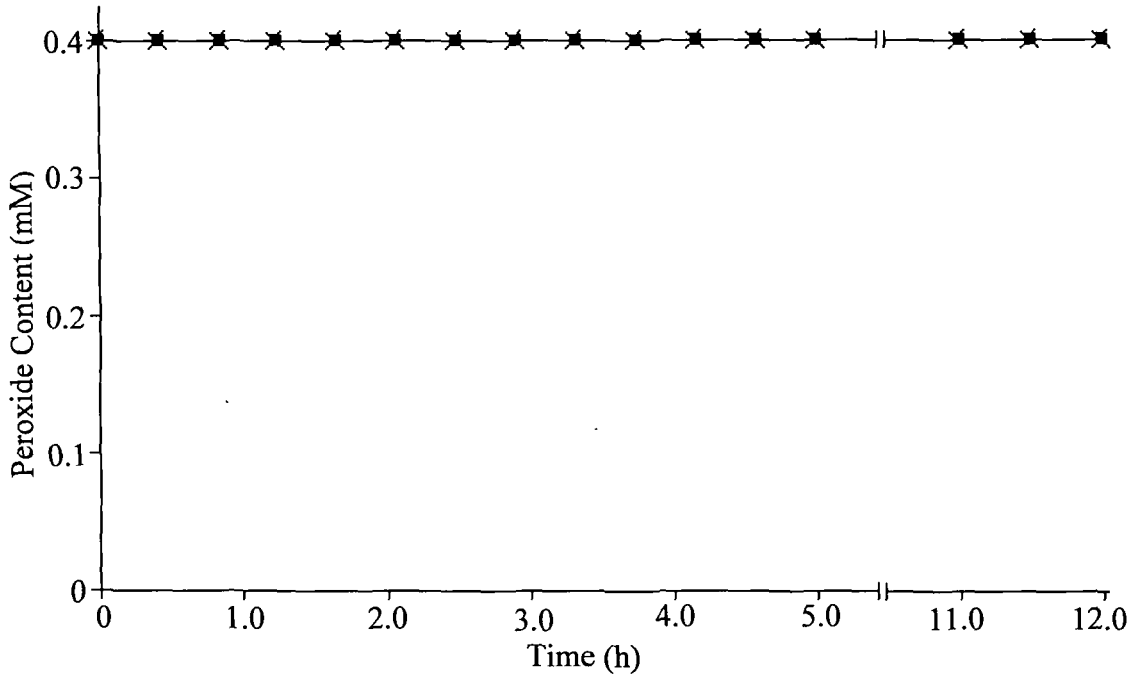


Fig. 3.30 Stability of compound **PAW (3.1)** at different pH values: (■) compound solution in distilled water, pH of the solution = 5.0, (×) solution of the compound in phosphate buffer (50 mM, pH 7.0).

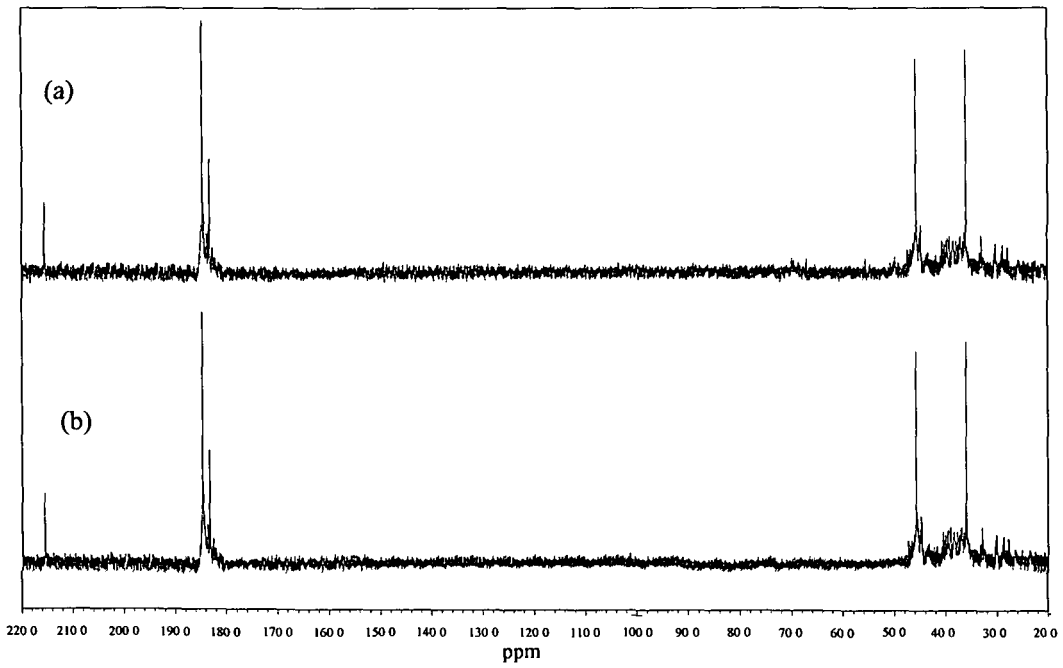


Fig. 3.31 ^{13}C NMR spectra of **PAW (3.1)** in D_2O, recorded (a) immediately after preparation and (b) solution of (a) 24 h later, showing stability of the compound in solution.

3.4 CONCLUSIONS

In conclusion, viable synthetic routes have been established to obtain a new series of water soluble, stable and structurally defined tungsten containing macromolecules by anchoring the pW species to appropriate polymer matrices. Moreover, synthesis of a heretofore unreported insoluble pW containing polymeric compound could be achieved by immobilization of the pW species on poly(acrylonitrile) matrix. The synthetic methodology developed, provides an efficient, straightforward pathway which opens opportunity to gain an easy access to peroxo derivatives anchored to soluble as well as insoluble polymers.

It is notable that pW moiety occurs in the first type of compounds in its oxodiperoxo form whereas in the latter complex it binds to the polymer as dioxomonoperoxo unit. An important finding of the present investigation, which may also be of clinical relevance is that the compounds retain their structural integrity in solution of a wide range of pH, particularly at acidic pH. This feature 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[79].

Results of investigation on the activity of the title compounds as catalyst or stoichiometric oxidant in organic oxidations, as well as the findings on their interaction with the enzyme catalase and phosphatases are presented in Chapters **4, 5, 6** and **7** of the thesis.

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CHAPTER 4

Polymer Immobilized Peroxotungsten Compounds as Efficient Catalysts for Selective Oxidation of Sulfides with Hydrogen Peroxide

4.1 INTRODUCTION

The contemporary interest in the selective oxidation of sulfur containing compounds, as emphasized in Chapter 1, has to a great extent been fuelled by the utility of sulfoxides as fine chemicals, pharmaceuticals and as valuable intermediates for the construction of chemically and biologically active molecules[1-8]. Moreover, oxidative desulfurization processes are emerging as important and sustainable procedures for obtaining ultra low sulfur fuels[9-15].

Aqueous H_2O_2 of less than 60% concentration has been recognized as ideal green oxidizing agent[16-18] for oxidation of sulfides in view of its high effective oxygen content, cleanliness (it produces water as the only by-product), safety in operation and storage[19]. However, owing to its being a mild oxidant the H_2O_2 mediated oxidations are usually very slow and often require to be activated by homogeneous or heterogeneous catalysts[19-29]. This feature has spurred the development of a plethora of useful and interesting catalysts for H_2O_2 mediated oxidation of sulfides, especially with the use of various types of tungsten based catalyst systems[19] such as the peroxotungsten complex [$WO(O_2)_2$, $HMPT/H_2O$],[30] tungstic oxide (WO_3),[31] tungstic acid [H_2WO_4],[32] hexacarbonyltungsten [$W(CO)_6$],[33] phosphotungstenic acid [$H_3(P(W_3O_{10})_4).H_2O$],[34] [$C_5H_5N(n\text{-}C_{16}H_{33})]_3PO_4[W(O)(O_2)_2]_4$,[35] Na_2WO_4 and other tungsten complexes with various quaternary ammonium salts as phase-transfer catalysts[36-39]. Nevertheless, scope for improvement still remains owing to some of the disadvantages associated with the

available protocols viz., over oxidation, low TON, high cost, difficulty in regeneration of the catalyst, and requirements such as high temperature, long reaction time, a promoter or a co-catalyst, chlorohydrocarbon solvents and excessive H_2O_2. Therefore, search for newer catalysts, active under improved environmentally acceptable conditions, for selective oxidation of sulfides continues unabated.

Keeping in view the aforementioned observations and in continuation of our work on peroxotungstates described in Chapter 3, in the present work, we focused on exploring the potential of the newly synthesized, soluble as well as insoluble polymer-immobilized peroxotungstate compounds, for their ability to serve as homogeneous or heterogeneous catalysts for selective oxidation of organic sulfide to sulfoxide or sulfone, under mild reaction conditions.

Anchoring of catalytically active transition metal complexes has been receiving 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[40-43]. 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. The task of establishing a robust polymer-metal bond which will be retained after repeated catalytic cycles is particularly challenging in the regeneration of supported catalytic species[40,44,45]. Polymer-immobilized peroxometallates are yet to be fully explored for their potential as environmentally benign heterogeneous catalysts in organic oxidations. Although catalysis is a relatively new area for application of water soluble polymers, nevertheless, as has been highlighted in Chapter 1 and 3, soluble linear polymers are increasingly being recognized as excellent candidates as reagent and catalyst support in organic chemistry[46-48]. Moreover, the use of more benign solvents such as water is now a subject of considerable interest in the context of "green" chemistry.

Here, we present the results of our investigation on the activity of the immobilized complex, **PANW (3.1)** as a versatile heterogeneous catalyst in selective oxidation of a range of thioethers and dibenzothiophene (DBT) with H_2O_2, to the corresponding sulfoxide or sulfone. The water soluble polymer anchored compounds, **3.2-3.5** served as homogeneous catalyst for the same reactions. The two classes of compounds enabled us to draw comparison on their efficiency as catalyst in the chosen reactions. A set of our results has also been compared with those of others obtained using W based catalysts to enable us comment on the efficacy of the protocol.

4.2 EXPERIMENTAL SECTION

4.2.1 General procedure for selective oxidation of sulfides to sulfoxides

In a representative procedure, organic substrate (5 mmol) was added to a mixture of catalyst (containing 0.005 mmol of W) [**PANW** (13.2 mg), **PAW** (3.06 mg), **PMAW** (4.00 mg) or **PAmW** (3.90 mg)] and 30% H_2O_2 (1.13 mL, 10 mmol) in methanol (5 mL), maintaining molar ratio of W:substrate at 1:1000 and substrate:H_2O_2 at 1:2, in a 50 mL two-necked round-bottomed flask. The reaction was conducted at room temperature (RT) under continuous stirring. The progress of the reaction was monitored by thin layer chromatography (TLC) and GC. After completion of the reaction, the product as well as unreacted organic substrates were extracted with diethyl ether and dried over anhydrous Na_2SO_4 and distilled under reduced pressure to remove excess diethyl ether. The corresponding sulfoxide obtained was purified by column chromatography on silica gel using ethyl acetate and n-hexane (1:9).

The products obtained were characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopy and in case of solid sulfoxide products, in addition to the above spectral analysis, we have also carried out melting point determination (see Appendix: 4A).

4.2.2 General procedure for selective oxidation of sulfides to sulfones

To a stirred solution of sulfide (5 mmol) in acetonitrile (5 mL), 50% H_2O_2 (1.36 mL, 20 mmol) and the catalyst (containing 0.005 mmol of W) [**PANW** (13.2 mg), **PAW** (3.06 mg), **PMAW** (4.00 mg) or **PAmW** (3.90 mg)] were added successively maintaining molar ratio of W:substrate at 1:1000 and substrate:H_2O_2 at 1:4. The resulting reaction mixture was stirred at room temperature. After completion of the reaction, the sulfone obtained was isolated, purified and characterized by methods similar to those mentioned under procedure for oxidation of sulfide to sulfoxide (Section 4.2.1).

4.2.3 Regeneration of catalyst

The regeneration of the catalyst for reuse was tested for the reaction using methyl phenyl sulfide (MPS) as substrate. Since we have optimized two types of reaction conditions for selective transformation of sulfide to sulfoxide or sulfone, the recyclability of the reagent was examined for the afore mentioned reactions separately. In the sulfoxidation reaction by **PANW** (**3.1**), the reaction mixture contained the same recipe mentioned under Section 4.2.1. The catalyst was separated by centrifugation after being used in the reaction (35 min) washed in acetone and dried in vacuo over concentrated sulfuric acid. The catalyst was then placed into a fresh reaction mixture containing 30% H_2O_2 (1.13 mL, 10 mmol), substrate (5 mmol) and methanol (5 mL). The progress of the reaction was monitored by thin layer chromatography (TLC) and GC.

In the **PANW (3.1)** catalyzed oxidation of MPS to sulfone, the separated catalyst after completion of the reaction (90 min) was similarly subjected to further catalytic cycle by placing it into a fresh reaction mixture of 50% H_2O_2 (1.36 mL, 20 mmol), MPS (5 mmol) and acetonitrile (5 mL) as mentioned under Section 4.2.2.

In an alternative procedure, regeneration of the spent catalyst, **PANW (3.1)** was achieved by charging the spent reaction mixture, remaining in the reaction vessel after separating the organic reaction product, with fresh H_2O_2 and substrate and then repeating the experiment. Each of the procedures was repeated for seven cycles.

In case of regeneration of homogeneous catalysts, **PAW (3.2), PMAW (3.3)** or **PAmW (3.4)**, the following method has been adopted by using MPS as substrate. For selective sulfoxidation, after completion of the reaction mentioned under Section 4.2.1, the organic reaction product as well as unreacted substrate was extracted from the reaction mixture by diethyl ether extraction. The aqueous portion of the reaction mixture containing the spent catalyst was treated with 30% H_2O_2 (1.13 mL, 10 mmol). Subsequently, a fresh lot of MPS (5 mmol) and methanol (5 mL) was added to it. The progress of the reaction was monitored by thin layer chromatography (TLC) and GC. The process was repeated for a total of three reaction cycles.

Similarly, after completion of the reaction of oxidation of MPS to sulfone mentioned under Section 4.2.2, the organic reaction product as well as unreacted substrate was extracted from the reaction mixture by diethyl ether extraction. The catalyst was regenerated by treating the spent reaction mixture with 50% H_2O_2 (1.36 mL, 20 mmol) followed by a fresh lot of MPS (5 mmol) and acetonitrile (5 mL). The progress of the reaction was monitored by thin layer chromatography (TLC) and GC. The reusability of the catalyst was examined for three reaction cycles.

4.3 RESULTS AND DISCUSSION

Catalytic performances of the synthesized pW compounds viz. the immobilized complex **PANW (3.1)** and water soluble polymer anchored complexes, **PAW (3.2), PMAW (3.3), PAmW (3.4),** as heterogeneous or homogeneous catalysts in the oxidation of organic sulfides, using H_2O_2 as terminal oxidant, has been explored.

4.3.1 The compound PANW (3.1) as a catalyst in sulfoxidation - optimization of reaction condition

In order to optimize the reaction conditions, several reaction runs were performed using methyl phenyl sulfide (MPS) as model substrate (S) in presence of different solvents as shown in Table 4.1.

The reactions were conducted at room temperature (RT) under magnetic stirring. In a preliminary experiment, MPS was allowed to react with H_2O_2 (1 equivalent) maintaining the W:substrate molar ratio at 1:1000 in methanol. Under these conditions MPS has been rapidly oxidized initially to sulfoxide and then to sulfone to afford ultimately a mixture of **1a** and **1b** in the ratio of 77:23 [Table 4.1, entry 1]. The initial fast reaction was observed to slow down on prolonged reaction time and remained incomplete even after 5 h. The significant finding of the reaction run was the facile formation of sulfoxide as the exclusive product within the initial period of *ca.* 30 minutes of starting the reaction. The observation led us to examine the possibility of achieving selective sulfoxidation of MPS by terminating the reaction at specific time after the initial formation of sulfoxide in order to prevent over oxidation to sulfone. Indeed, when a reaction carried out separately under analogous condition was terminated after 35 minutes

of its starting, pure sulfoxide could be isolated in *ca.* 49% yield [Table 4.1, entry 2]. The yield of **1a** could be improved further to nearly 72% by increasing the amount of H_2O_2 to 2 equivalents, leading to reasonably high TOF [Table 4.1, entry 3]. A 1:2 molar ratio of substrate:oxidant, thus appeared to be optimal in order to achieve high TOF without affecting the selectivity. Allowing a similar reaction to run for 50 minutes although led to complete conversion of sulfide with a nearly 97% isolated product yield [Table 4.1, entry 4], however, 16-20% of sulfone was also formed along with sulfoxide, thereby reducing the selectivity of the oxidation. The data obtained thus evidenced for higher selectivity at lower conversions.

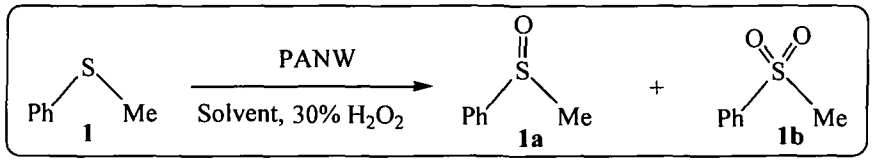
The effect of catalyst amount was then evaluated [Table 4.1, entries 5 and 6]. A 5 or 10 fold increase in the amount of catalyst, albeit led to an enhancement in the rate of the reaction. However, the corresponding TOF was found to decrease. We have therefore decided to maintain a W:S molar ratio at 1:1000 for subsequent reactions.

In a control experiment conducted in absence of the catalyst, very little conversion of MPS to sulfoxide or sulfone was observed (<10%) under similar reaction condition. It is thus evident that the catalyst plays a crucial role in facilitating the desired reactions.

4.3.1.1 Effect of nature of solvent and temperature

The nature of the solvent was found to play a crucial role in determining the activity and selectivity of the catalyst in the oxidation reactions. We have carried out the reactions using environmentally acceptable common organic solvents such as methanol,

Table 4.1 Optimization of reaction conditions for **PANW** catalyzed selective oxidation of methyl phenyl sulfide (MPS) by 30% H_2O_2[a]



Entry	Molar ratio W:MPS	H_2O_2 (equiv.)	Solvent	Temperature	Time (min)	Isolated Yield (%)	**1a : 1b**	TOF (h^{-1})
1	1:1000	1	MeOH	RT	300	79	77 : 23	158
2	1:1000	1	MeOH	RT	35	49	100 : 0	840
3	**1:1000**	**2**	**MeOH**	**RT**	**35**	**72**	**100 : 0**	**1235**
4	1:1000	2	MeOH	RT	50	97	84 : 16	1164
5	1:500	2	MeOH	RT	20	73	100 : 0	1095
6	1:100	2	MeOH	RT	10	71	100 : 0	426
7	1:1000	2	EtOH	RT	35	70	100 : 0	1200
8	1:1000	2	$CHCl_3$	RT	120	7	87:13	35
9	1:1000	2	CH_2Cl_2	RT	120	11	84:16	55
10	1:1000	2	MeCN	RT	120	96	46 : 54	480
11	1:1000	2	MeCN	RT	45	67	75 : 25	893
12	1:1000	2	MeOH	50 °C	15	73	100 : 0	2920
13	1:1000	2	MeOH	65 °C	10	74	100 : 0	4440

[a]All reactions were carried out with 5 mmol of substrate in 5 mL of solvent.

ethanol and acetonitrile, fully miscible with the organic substrate and H_2O_2, as well as in water. The data in Table 4.1 clearly shows that methanol is highly effective as a solvent affording both product selectivity as well as high TOF in presence of the catalyst. Ethanol was found to be equally effective for the reaction indicating a favorable effect of the protic solvent [Table 4.1, entry 7]. The observation is in agreement with previous report that solvents of high hydrogen bonding ability favor the formation of sulfoxide with high chemoselectivity[49-51]. No appreciable conversion was noted in aqueous medium in the present case probably due to the insolubility of the catalyst as well as the substrate in water. Reactions were also conducted in solvents such as chloroform or dichloromethane which yielded low conversions of MPS within the range of 7-11% after 2 h of reaction time[Table 4.1, entries 8 and 9]. It is interesting to note that when the reaction was conducted in acetonitrile under analogous condition, the concomitant formation of the

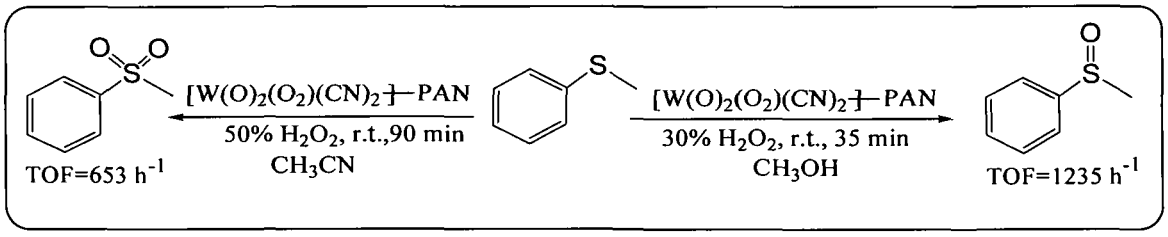


Fig. 4.1 Optimized conditions for selective oxidation of methyl phenyl sulfide to sulfoxide or sulfone catalyzed by **PANW (3.1)**

time[Table 4.1, entries 8 and 9]. It is interesting to note that when the reaction was conducted in acetonitrile under analogous condition, the concomitant formation of the corresponding sulfone was observed along with sulfoxide [Table 4.1, entries 10 and 11]. Therefore, selective sulfoxidation of MPS could not be attained in acetonitrile even by stopping the reaction at lower conversion.

It is noteworthy that on increasing the reaction temperature upto 65 °C, a 3-4 fold increase in TOF could be achieved without affecting the selectivity [Table 4.1, entries 12 and 13]. This striking feature of the protocol indicated its synthetic utility.

4.3.2 The compound PAW (3.2) as a catalyst - optimization of reaction condition for sulfoxidation

The conditions of reaction such as reaction temperature, substrate:oxidant stoichiometry, catalyst concentration and type of solvent were similarly optimized using methyl phenyl sulfide (MPS) as model substrate and **PAW (3.2)** as a representative (Table 4.2). The reaction of the substrate with 30% H_2O_2 in the molar ratio of 1:2 in presence of the catalyst (catalyst:substrate molar ratio of 1:1000) in methanol proceeded smoothly to yield selectively sulfoxide within the initial period of *ca.* 1 h of starting the reaction with nearly 75% yield. These conditions are identical to the reaction conditions optimized for the **PANW (3.1)** catalyzed reaction. On increasing the reaction time to 90 minutes, nearly 100% conversion of the substrate occurred. However, the product obtained was a mixture of **1a** and **1b** [Table 4.2, entry 4]. Therefore, in order to achieve 100% selectivity with respect to sulfoxide, the reactions were required to be terminated at 70-75% conversion within 50-55 minutes of starting the reaction.

Table 4.2 Optimization of reaction conditions for **PAW** catalyzed selective oxidation of methyl phenyl sulfide (MPS) by 30% H_2O_2[a]

Entry	Molar ratio W:MPS	H_2O_2 (equiv.)	Solvent	Temperature	Time (min)	Isolated Yield (%)	**1a : 1b**	TOF (h^{-1})
1	1:1000	1	MeOH	RT	300	71	82 : 18	142
2	1:1000	1	MeOH	RT	55	46	100 : 0	501
3	**1:1000**	**2**	**MeOH**	**RT**	**55**	**73**	**100 : 0**	**796**
4	1:1000	2	MeOH	RT	90	98	87 : 13	653
5	1:500	2	MeOH	RT	35	74	100 : 0	634
6	1:100	2	MeOH	RT	25	68	100 : 0	163
7	1:1000	2	EtOH	RT	55	72	100 : 0	785
8	1:1000	2	$CHCl_3$	RT	120	8	91:9	40
9	1:1000	2	CH_2Cl_2	RT	120	10	88:12	50
10	1:1000	2	MeCN	RT	180	97	48 : 52	323
11	1:1000	2	MeCN	RT	65	69	79 : 21	636
12	1:1000	2	MeOH	50 °C	30	70	100 : 0	1400
13	1:1000	2	MeOH	65 °C	15	72	100 : 0	2880

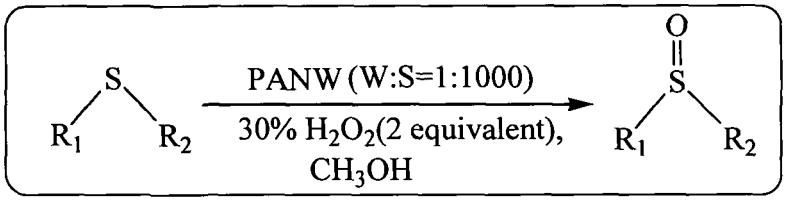
[a]All reactions were carried out with 5 mmol of substrate in 5 mL of solvent.

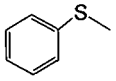
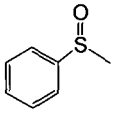
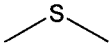
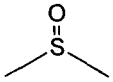
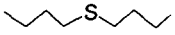
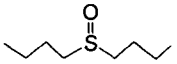
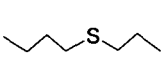
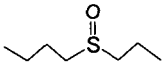
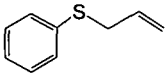
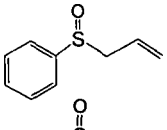
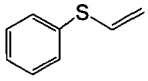
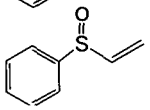
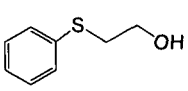
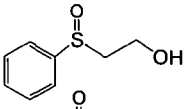
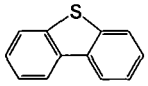
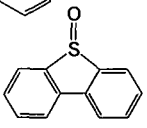
4.3.3 Selective oxidation of sulfides to sulfoxides by H_2O_2 - PANW (3.1), PAW (3.2), PMAW (3.3) or PAmW (3.4) as catalysts

Having optimized the right conditions for sulfoxidation, a series of structurally diverse sulfides were subjected to the oxidation reaction by H_2O_2, in presence of the catalysts **PANW (3.1), PAW (3.2), PMAW (3.3)** or **PAmW (3.4)**. The data presented in Table 4.3 and 4.4 show that all the catalysts, irrespective of being heterogeneous or homogeneous, catalyzed the selective oxidation of aliphatic and aromatic sulfides as well as DBT to the corresponding sulfoxide under the optimum condition, in impressive yields and TOFs. A comparison between the catalytic activity of the immobilized compound **PANW (3.1)** and its homogeneous analogues showed the higher efficiency of the insoluble **PANW (3.1)**. For instance, heterogeneous catalyst **PANW (3.1)** exhibited the highest TOF of 1235 h^{-1} at room temperature for the sulfoxidation of MPS which is nearly double in magnitude compared to the TOF obtained for the catalyst **PAW (3.2)**. Further, in order to evaluate the efficacy of the catalyst, we have compared the results of our investigation involving reaction of MPS and H_2O_2 with those of a few other tungsten based catalysts (Table 4.5). The data obtained revealed that polymer supported catalyst **PANW (3.1)** as well as the soluble catalysts displayed superior activity in terms of TOF as well as selectivity, achieved under mild reaction condition, over majority of tungsten containing catalysts reported for sulfide oxidation (Table 4.5).

Significantly, the maximum selectivity of 100% for all the catalysts was achieved at 70-75% of conversion for the substrates [Table 4.3 and 4.4, entries 1-8]. Termination of the reactions at these conversions thus afforded sulfoxides of high purity. It is pertinent to mention here that no trace of sulfone was detected in the isolated products at this stage of the reactions. The conversion:selectivity as a function of time was investigated for each of the substrates using **PANW (3.1)** as catalyst (Table 4.6). Although increased conversions

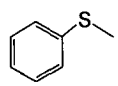
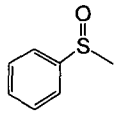
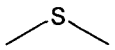
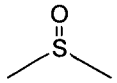
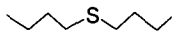
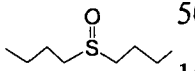
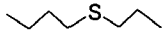
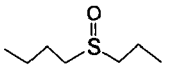
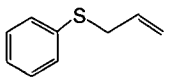
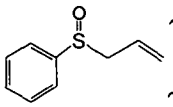
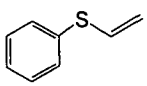
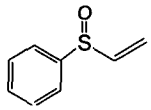
Table 4.3 Selective oxidation of sulfides to sulfoxides with 30% H_2O_2 using **PANW** as catalyst[a] at room temperature or 65 °C[b] (values within parenthesis)



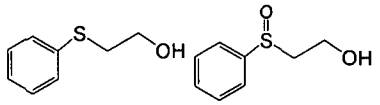
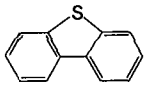
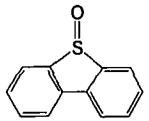
Entry	Substrate	Time (min)	Product	Isolated Yield (%)	TOF [c] (h^{-1})
1		35 (10)		72 (74)	1235 (4440)
		35 (10)[d]		69 (72)	1182 (4320)
		35 (10)[e]		73 (70)	1251 (4200)
2		30		71	1420
3		30 (10)		72 (75)	1440 (4500)
4		30 (10)		68 (74)	1360 (4440)
5		45 (12)		65 (70)	866 (3500)
6		180 (55)		76 (74)	253 (807)
7		70 (20)		74 (76)	634 (2280)
8[f]		(420)		(71)	(10)

[a]Reaction conditions: substrate (5 mmol), catalyst (13.2 mg, 0.005 mmol of W), 30% H_2O_2 (1.13 mL, 10 mmol), methanol (5 mL).
[b]Reaction at 65 °C in refluxing methanol.
[c]TOF (Turnover frequency) = mmol of product per mmol of catalyst per hour.
[d]Yield of 7th reaction cycle.
[e]Yield at 5 g scale.
[f]Substrate (5 mmol), catalyst (132 mg, 0.05 mmol of W), methanol (5 mL).

Table 4.4 Selective oxidation of sulfides to sulfoxides with 30% H_2O_2 catalyzed by **PAW, PMAW** and **PAmW**[a]

Entry	Substrate	Product	PAW			PMAW			PAmW		
			Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})	Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})	Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})
1			55	73	796	65	72	664	70	70	600
			15[c]	72	2880	20[c]	74	2220	25[c]	73	1752
			55	36[d]	392	65	34[d]	313	70	33[d]	282
			15[c]	35[d]	1400	20[c]	31[d]	930	25[c]	29[d]	696
			55	74[e]	807	65	71[e]	655	70	72[e]	617
			15[c]	71[e]	2840	20[c]	70[e]	2100	25[c]	69[e]	1656
2			45	72	960	50	69	828	60	71	710
3			50	73	876	55	75	818	65	72	664
			15[c]	71	2840	17[c]	72	2541	25[c]	70	1680
4			50	72	864	55	73	796	65	69	636
			15[c]	75	3000	17[c]	72	2541	25[c]	73	1752
5			70	72	617	75	69	552	85	71	501
			20[c]	68	2040	25[c]	70	1680	35[c]	66	1131
6			210	74	211	230	75	195	240	71	177
			55[c]	76	829	65[c]	71	655	70[c]	74	634

Continued...

Entry	Substrate	Product	PAW			PMAW			PAmW		
			Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})	Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})	Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})
7			90	73	486	100	71	426	120	70	350
			30[c]	74	1480	35[c]	68	1165	40[c]	71	1065
8[f]			7.5[c]	70	9	8.0[c]	72	9	8.0[c]	68	8

[a]All reactions were carried out with 5 mmol substrate, 10 mmol 30% H_2O_2 and catalyst (0.005 mmol of W) in 5 mL methanol at RT, unless otherwise indicated.

[b]TOF (Turnover frequency) = mmol of product per mmol of catalyst per hour.

[c]Reaction at 65 °C in refluxing methanol.

[d]Yield of 3rd reaction cycle.

[e]Yield at 5 g scale.

[f]Substrate (5 mmol), catalyst (0.05 mmol of W), methanol (5 mL).

were noted with increasing reaction time for each of the substrates tested however, the selectivity was found to be reduced due to the expected overoxidation to sulfone. The observation is consistent with the earlier findings of Choudary and co-workers[52] pertaining to activity of a W based LDH catalyst in sulfide oxidation.

The rate of the oxidation of thioethers were observed to vary depending on the nature of the substrates and the substituents attached [Table 4.3 and 4.4, entries 1-8]. Allylic and vinylic sulfides [Table 4.3 and 4.4, entries 5 and 6] were found to be less readily oxidized by H_2O_2 than the dialkyl sulfides probably due to effect of conjugation. These observations are in agreement with the earlier findings that rate of oxidation of sulfides by H_2O_2 increases with the increased nucleophilicity of the sulfide[52,59]. The present protocol is also effective for much less nucleophillic sulfide, such as DBT. Significantly, selective oxidation of DBT, a refractory sulfide, to sulfoxide has been reported to be difficult to achieve with most of the available reaction procedures[14,16,59,60]. In the present work a moderately higher temperature of 65 ^{o}C was required to be employed in case of DBT oxidation since the reaction was relatively sluggish at room temperature. Also, a higher amount of catalyst used (W:substrate ratio of 1:100) to further enhance the rate of the reaction [Table 4.3 and 4.4, entry 8].

Each of the catalysts exhibited excellent chemoselectivity toward sulfur group of substituted sulfides containing other oxidation prone functional groups such as –C=C– and –OH [Table 4.3 and 4.4, entries 5-7]. Allylic and vinylic sulfoxides were obtained without epoxidation products under such conditions.

It is pertinent to highlight herein that the methodology worked well for a relatively larger scale oxidation using 5 g of MPS to give sulfoxide [Table 4.3 and 4.4, entry 1^{e}] in high yields, proving its potential for scaled-up synthetic applications.

Table 4.5 Oxidation of thioanisol catalyzed by different tungsten based catalysts - comparison with catalytic performance of **PANW** and **PAW**

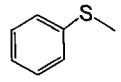
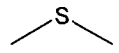
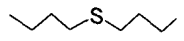
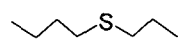
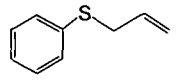
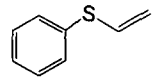
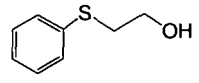
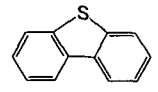
Entry	Catalyst	Solvent	Time (h)	Conversion(%) / Yield (%)	**1a:1b**	TOF (h^{-1})	Reference
1	**PANW (3.1)**	MeOH	0.58	72	100:0	1235	
2	**PANW (3.1)**	CH_3CN	1.50	98	0:100	653	
3	**PAW (3.2)**	MeOH	0.91	73	100:0	796	
4	**PAW (3.2)**	CH_3CN	2.5	97	0:100	388	
5	LDH-WO_4^{2-}	H_2O	0.5	94	88:12	42.72	52
6	$NaWO_4$	H_2O	2.5	95	70:30	9	52
7	LDH-{$PO_4[WO(O_2)]_4$}	H_2O	4.0	90	40:60	15	52
8	Silica-Based Ammonium Tungstate	DCM:MeOH	1.5	82	100:0	27.33	20
9	Na_2WO_4,$C_6H_5PO_4H_2$ and PTC {PTC= $[CH_3(n\text{-}C_8H_{17})_3N]HSO_4$}	PTC	2	97	0:100	485	53
10	Na_2WO_4,$C_6H_5PO_4H_2$ and PTC {PTC= $[CH_3(n\text{-}C_8H_{17})_3N]HSO_4$}	PTC	9	93	100:0	222	53
11	WO_3–cinchona alkaloids	THF	49	88	----	0.35	54
12	Nanosized WO_3 particle supported on MCM-48	MeOH	12	99	0:100	8.25	55
13	Nanosized WO_3 particle supported on MCM-48	MeOH	4	97	100:0	24.25	55

Continued...

Entry	Catalyst	Solvent	Time (h)	Conversion(%) / Yield (%)	**1a:1b**	TOF (h^{-1})	Reference
14	$[NMe_4]_2[(PhPO_3)\{WO(O_2)_2\}_2\{WO(O_2)_2(H_2O)\}]EtOH$	CH_3Cl	2	100	3:97	100	56
15	$[NBu_4][(Ph_2PO_2)\{WO(O_2)_2\}_2]$	CH_3Cl	2	100	2:98	100	56
16	Phosphotungstic acid ($H_3PW_{12}O_{40}$) supported on a non cross-linked copolymer of N-isopropylacrylamide with an ammonium	Solventless	4	100	3:98	125	57
17	$[W_2O_3(O_2)_4]^{2-}$ immobilized on ionic liquid-modified silica (SiO_2-W_2-Im)	MeOH:DCM	2.5	97.9	100:0	26.10	58
			5	96.3	0:100	9.63	58
18	$[W_2O_3(O_2)_4]^{2-}$ immobilized on ionic liquid-modified silica (SiO_2-W_2-Py)	MeOH:DCM	2.5	95.6	100:0	25.49	58
			5	90.4	0:100	9.04	58
19	W-LDH (WO_4^{2-})	CH_3CN	0.5	98.0	15:85	14.0[a]	59
20	PW-LDH ($W_7O_{24}^{6-}$)	CH_3CN	0.5	93.0	17:83	13.1[a]	59

[a] mol $l^{-1}h^{-1}g_{cat}^{-1}$

Table 4.6 Conversion/selectivity as a function of time in **PANW** catalyzed[a] selective sulfoxidation with 30% H_2O_2

Entry	Substrate	Time (min)	Isolated Yield (%)	Selectivity Sulfoxide:Sulfone
1		35	72	100:0
		50	97	85:15
2		30	71	100:0
		40	95	83:17
3		30	72	100:0
		45	98	84:16
4		30	71	100:0
		45	97	86:14
5		45	65	100:0
		60	96	79:21
6		180	76	100:0
		240	98	85:15
7		70	74	100:0
		90	94	83:17
8[b]		420	71	100:0
		480	93	86:14

[a]Reaction conditions: substrate (5 mmol), catalyst (13.2 mg, 0.005 mmol of W), 30% H_2O_2 (1.13 mL, 10 mmol), methanol (5 mL), RT.

[b]Substrate (5 mmol), catalyst (132 mg, 0.05 mmol of W), methanol (5 mL), 65 °C.

4.3.4 Oxidation of sulfides to sulfones

It has been observed that formation of sulfone is favored along with sulfoxide when oxidation of MPS was carried out in acetonitrile [Table 4.1, entries 10 and 11] using **PANW (3.1)** as catalyst. This feature was found to be common for the soluble catalyst **PAW (3.2)** as well [Table 4.2, entries 10 and 11]. We have therefore decided to explore the possibility of achieving selective oxidation of sulfides to sulfones by conducting the pW catalysed reactions in acetonitrile. Our initial attempts to obtain pure sulfone using MPS as a model substrate were however, unsuccessful when we used 30% H_2O_2 as the oxidant. Even at a high substrate:oxidant molar ratio of 1:5 a mixture of **1a** and **1b** were obtained. On the other hand, clean conversion to sulfone could be achieved for MPS, in excellent yield, and at room temperature within a reasonably short reaction time by replacing 30% H_2O_2 with 50% H_2O_2 and maintaining the W:S ratio at 1:1000. It is evident from the data presented in Tables 4.7 and 4.8 that the selectivity towards sulfone gradually increases with increasing concentration of H_2O_2. For both the catalysts, a substrate:oxidant molar ratio of 1:4 was optimum for the complete conversion of MPS into sulfone under the reaction conditions used [Table 4.7 and 4.8, entry 4]. Apart from MPS, the protocol was also found to be effective for a wide range of aromatic and aliphatic substrates, the latter being more reactive as expected. Subsequently, the efficacy of the other soluble pW complexes in selective oxidation of sulfide to sulfone was assessed under the optimized condition. The results are summarized in Table 4.7 and 4.8. The water soluble supported complexes although were observed to be effective catalyst, appears to be less efficient compared to **PANW (3.1)** under identical optimized conditions.

Table 4.7 Optimization of reaction conditions for **PANW (3.1)** catalyzed selective oxidation of methyl phenyl sulfide (MPS) to sulfone by 50% H_2O_2[a]

Entry	Molar ratio W:MPS	H_2O_2 (equiv.)	Solvent	Temperature	Time (min)	Isolated Yield (%)	**1a : 1b**	TOF (h^{-1})
1	1:1000	1	MeCN	RT	240	55	46 : 54	137
2	1:1000	2	MeCN	RT	180	97	37 : 63	323
3	1:1000	3	MeCN	RT	120	96	13 : 87	480
4	**1:1000**	**4**	**MeCN**	**RT**	**90**	**98**	**0 : 100**	**653**
5	1:500	4	MeCN	RT	60	99	0 : 100	495
6	1:100	4	MeCN	RT	40	97	0 : 100	145
7	1:1000	4	MeCN	78 °C	25	96	0 : 100	2304

[a]All reactions were carried out with 5 mmol of substrate in 5 mL of solvent.

Table 4.8 Optimization of reaction conditions for **PAW (3.2)** catalyzed selective oxidation of methyl phenyl sulfide (MPS) to sulfone by 50% H_2O_2[a]

Entry	Molar ratio W:MPS	H_2O_2 (equiv.)	Solvent	Temperature	Time (min)	Isolated Yield (%)	**1a : 1b**	TOF (h^{-1})
1	1:1000	1	MeCN	RT	360	59	49 : 51	98
2	1:1000	2	MeCN	RT	270	94	33 : 67	208
3	1:1000	3	MeCN	RT	210	96	18 : 82	274
4	**1:1000**	**4**	**MeCN**	**RT**	**150**	**97**	**0 : 100**	**388**
5	1:500	4	MeCN	RT	100	95	0 : 100	285
6	1:100	4	MeCN	RT	60	98	0 : 100	98
7	1:1000	4	MeCN	78 °C	45	99	0 : 100	1320

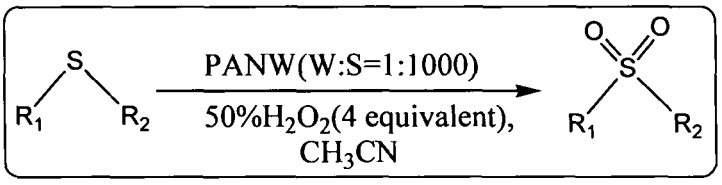
[a]All reactions were carried out with 5 mmol of substrate in 5 mL of solvent.

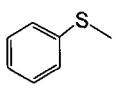
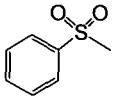
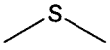
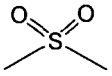
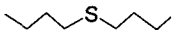
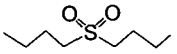
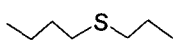
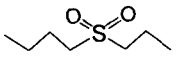
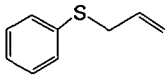
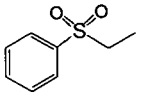
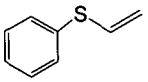
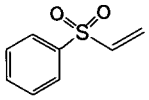
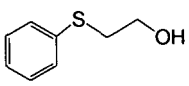
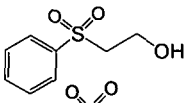
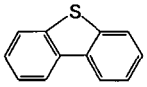
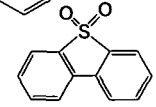
Most importantly, the TOF of the conversions could be substantially increased by carrying out the reactions under reflux at 78 °C. Even under such relatively drastic conditions viz., presence of reasonable excess of 50% H_2O_2 and high temperature, the co-existing alcohol and olefinic moieties in substituted sulfides remained unaffected [Table 4.9 and 4.10, entries 5-7]. The results presented in Table 4.9 and 4.10 show that the oxidation of sulfides to the corresponding sulfone has been achieved with complete chemoselectivity [Table 4.9 and 4.10, entries 5-7]. The suitability of the methodology for scaled up synthesis was also ascertained by conducting the reaction at a 5 g scale [Table 4.9 and 4.10, entry 1[e]].

4.3.5 Test for heterogeneity of the PANW (3.1) catalyzed reaction

In order to examine the leaching of the metal complex from the polymer –bound catalyst into the reaction medium during the oxidation reactions, separate experiments were carried out using MPS as the substrate. After completion of the reaction, the catalyst was separated by filtration. The filtrate collected was transferred to a reaction vessel and the reaction was allowed to continue for another 2 h, by adding a fresh MPS–H_2O_2 mixture. In the absence of the catalyst, under analogous conditions the sulfide conversion was noted to be *ca.* 5%, in line with the value obtained in absence of any catalyst. This demonstrates that the reaction did not proceed on the removal of the solid catalyst. These data are in agreement with the absence of catalyst leaching and the occurrence of a purely heterogeneous catalytic process.

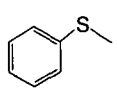
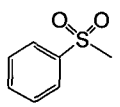
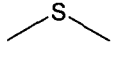
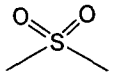
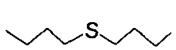
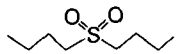
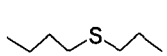
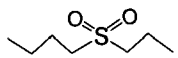
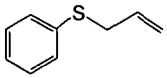
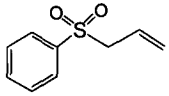
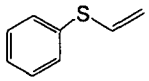
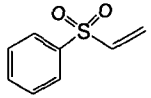
Table 4.9 Oxidation of sulfides to sulfones using 50% H_2O_2 catalyzed by **PANW**[a] at room temperature or 78 °C[b] (values within parenthesis)



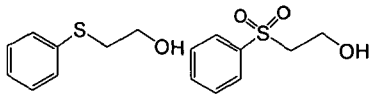
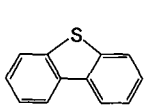
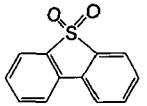
Entry	Substrate	Time (min)	Product	Isolated Yield (%)	TOF[c] (h^{-1})
1		90 (25)		98 (96)	653 (2304)
		90 (25)[d]		93 (91)	620 (2184)
		90 (25)[e]		94 (97)	626 (2328)
2		72		96	800
3		85 (25)		97 (98)	684 (2352)
4		85 (25)		95 (97)	670 (2328)
5		100 (30)		96 (98)	576 (1960)
6		360 (130)		95 (97)	158 (447)
7		270 (80)		96 (98)	213 (735)
8[f]		(570)		(93)	(9)

[a]Reaction conditions: substrate (5 mmol), catalyst (13.2 mg, 0.005 mmol of W), 50% H_2O_2 (1.36 mL, 20 mmol), acetonitrile (5 mL).
[b]Reaction at 78 °C in refluxing acetonitrile.
[c]TOF (Turnover frequency) = mmol of product per mmol of catalyst per hour.
[d]Yield of 7th reaction cycle.
[e]Yield at 5 g scale.
[f]Substrate (5 mmol), catalyst (132 mg, 0.05 mmol of W), acetonitrile (5 mL).

Table 4.10 Selective oxidation of sulfides to sulfones with 50% H_2O_2 catalyzed by **PAW, PMAW** and **PAmW**[a]

Entry	Substrate	Product	PAW			PMAW			PAmW		
			Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})	Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})	Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})
1			150	97	388	160	98	367	180	98	326
			45[c]	99	1320	55[c]	97	1058	60[c]	96	960
			150	57[d]	228	160	55[d]	206	180	58[d]	193
			45[c]	55[d]	733	55[c]	57[d]	621	60[c]	53[d]	530
			150	96[e]	384	160	95[e]	345	180	95[e]	316
			45[c]	98[e]	1306	55[c]	98[e]	1069	60[c]	97[e]	970
2			120	96	480	140	98	420	160	97	363
3			140	96	411	150	95	380	170	98	345
			45[c]	98	1306	50[c]	97	1164	55[c]	97	1058
4			140	97	415	150	95	380	170	96	338
			45[c]	95	1266	50[c]	96	1152	55[c]	98	1069
5			180	96	320	190	97	306	210	93	265
			55[c]	93	1014	65[c]	95	876	80[c]	95	712
6			420	96	137	440	94	128	450	93	124
			135[c]	97	431	150[c]	96	384	180[c]	95	316

Continued...

Entry	Substrate	Product	PAW			PMAW			PAmW		
			Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})	Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})	Time (min)	Isolated Yield (%)	TOF[b] (h^{-1})
7			300	95	190	320	94	176	330	96	174
			100[c]	93	558	110[c]	96	523	120[c]	95	475
8[f]			10.0[c]	92	9	11.0[c]	95	8	12.0[c]	91	7

[a]All reactions were carried out with 5 mmol substrate, 20 mmol 50% H_2O_2 and catalyst (containing 0.005 mmol of W) in 5 mL acetonitrile at RT, unless otherwise indicated.

[b]TOF (Turnover frequency) = mmol of product per mmol of catalyst per hour.

[c]Reaction at 78 °C in refluxing acetonitrile.

[d]Yield of 3rd reaction cycle.

[e]Yield at 5 g scale.

[f]Substrate (5 mmol), catalyst (0.05 mmol of W), acetonitrile (5 mL).

4.3.6 Catalyst recycling

The recyclability of the catalysts was assessed by using MPS as the substrate. The heterogeneous catalyst **PANW (3.1)** afforded regeneration and could be reused without further conditioning after separating the oxidized product and unreacted sulfide from the reaction mixture. The regeneration was accomplished by charging the spent catalyst with H_2O_2, a fresh lot of substrate and the respective solvent after each cycle of reaction. The catalyst was reused for upto seven catalytic cycles. Data presented in Table 4.3 [entry 1^d] and Table 4.9 [entry 1^d] shows that catalysts remain effective with consistent activity and selectivity even after seventh cycle of oxidation. The finding further indicates that the catalyst **PANW (3.1)** remains stable after several cycles of oxidations.

It is important to note that the overall TOF after seven catalytic cycles of oxidation of MPS was recorded to be 8,671 h^{-1} and 4,431 h^{-1} for sulfoxide and sulfone, respectively at room temperature. These values could further be increased to 30,300 h^{-1} (for sulfoxide) and 15,792 h^{-1} (for sulfone) by increasing the reaction temperature (Table 4.3, entry 1^d and Table 4.9, entry 1^d).

The water soluble catalysts could be regenerated in situ after separating the oxidized product and unreacted sulfide from the reaction mixture by extraction with ether. The regeneration was achieved by treating the spent reaction mixture containing the soluble catalyst with H_2O_2, a fresh lot of substrate and the respective solvent after completion of each cycle of reaction. However, unlike in the case of **PANW (3.1)** which showed consistent activity even after seven reaction cycles, the homogeneous analogues showed a slight decrease in TOF with every increasing number of cycles. This clearly indicates loss of catalytic activity of the compounds due to the possibility of leaching of the active pW species from the polymer matrix.

4.3.7 Nature of the spent catalyst

The IR spectrum of the recovered catalyst **PANW (3.1)** was identical with the corresponding spectrum of the original starting complex showing the presence of peroxo group, terminal oxo and co-ordinated as well as free nitrile groups indicating that tungsten centres are intact and the coordination environments were not altered during the catalytic process. Though a slight loss in peroxide content was noted, however, the EDX analysis showed no significant decrease in the metal loading value of the spent catalyst compared to the original catalyst. These findings further indicate that the catalyst **PANW (3.1)** remains stable after several cycles of oxidations.

In case of the soluble catalysts too no appreciable change was noted in the IR spectra of the spent catalyst isolated from the reaction mixture by treating with acetone, compared to the corresponding IR of the original compound. However, gradual loss of pW species was confirmed by the elemental analysis of the spent catalyst which showed decrease in tungsten content.

4.3.8 The proposed mechanism

The schemes of reactions, shown in Fig. 4.2 and 4.3, are proposed which satisfactorily describes the principal features of our results. In case of the **PANW (3.1)** catalysed reaction (Fig. 4.2) it is plausible that the polymer-bound monoperoxotungsten species **I** reacts with H_2O_2 to yield the reactive diperoxotungsten intermediate **II** (reaction a). The electrophilicity of peroxotungstate intermediate being much higher than that of H_2O_2[55], it is reasonable to expect that facile transfer of electrophilic oxygen to the substrate **III** would take place to yield sulfoxide **IV** (reaction b) with concomitant

regeneration of the original monoperoxo W(VI) catalyst. The sulfoxide may further react with the active diperoxotungstate species **II** (reaction c) generated from another cycle of reaction between catalyst **I** and H_2O_2 (reaction a) to yield sulfone **V**. The reaction leading to the formation of sulfone thus appears to be a typical two step process. The facile oxidation of nucleophilic sulfide to sulfoxide is apparently an easier process than the second oxidation of the resulting less nucleophilic sulfoxide to sulfone[52,53]. Although further studies are necessary to establish the exact identity of the reactive diperoxotungsten(VI) species **II**, support for the proposed mechanism comes from the earlier findings that for a peroxotungstate species to be catalytically active in oxidation, an oxo-diperoxo configuration may be a pre-requisite[61-63].

In case of the homogeneous catalysts with reactive diperoxotungsten moieties, transfer of electrophilic oxygen from the species **I** (Fig 4.3) to the substrate **III** is likely to occur as a first step of the reaction generating the monoperoxotungsten intermediate **II** and sulfoxide **IV**. Sulfoxide may subsequently react with another mole of peroxotungstate to yield sulfone **V**. In presence of the terminal oxidant H_2O_2 the monoperoxo intermediate **II** combines with peroxide to regenerate the starting diperoxotungstate complex **I**.

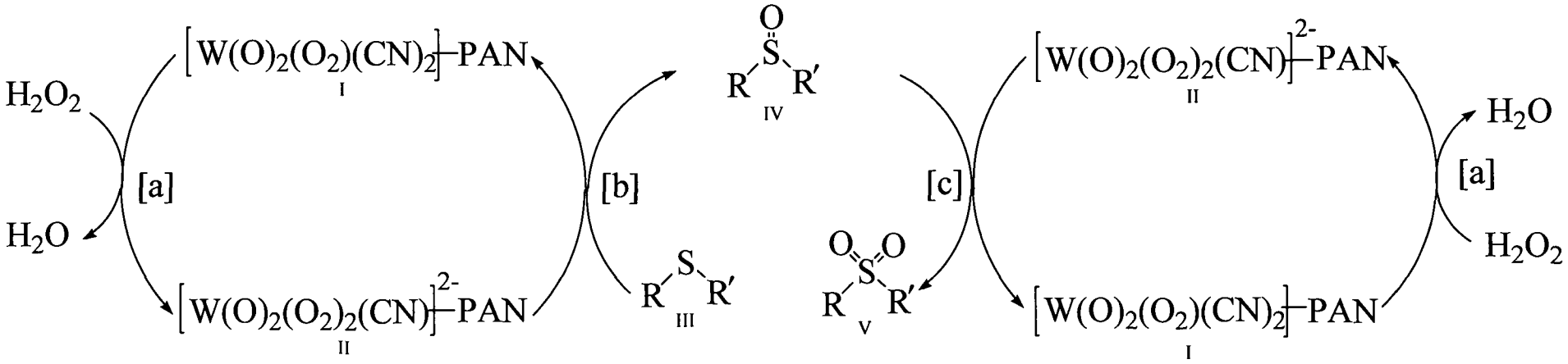


Fig 4.2. Schematic representation of reactions occurring with heterogeneous catalysts **PANW** (**3.1**). [a] The polymer-bound monoperoxotungsten species **I** reacts with H_2O_2 to yield the reactive diperoxotungsten intermediate **II**. [b] Transfer of electrophilic oxygen from **II** to the substrate **III** takes place to yield sulfoxide **IV** with concomitant regeneration of the original catalyst **I**. [c] The sulfoxide **IV** may further react with active diperoxotungstate species **II** leading to the formation of sulfone **V** and simultaneous regeneration of the original catalyst **I**.

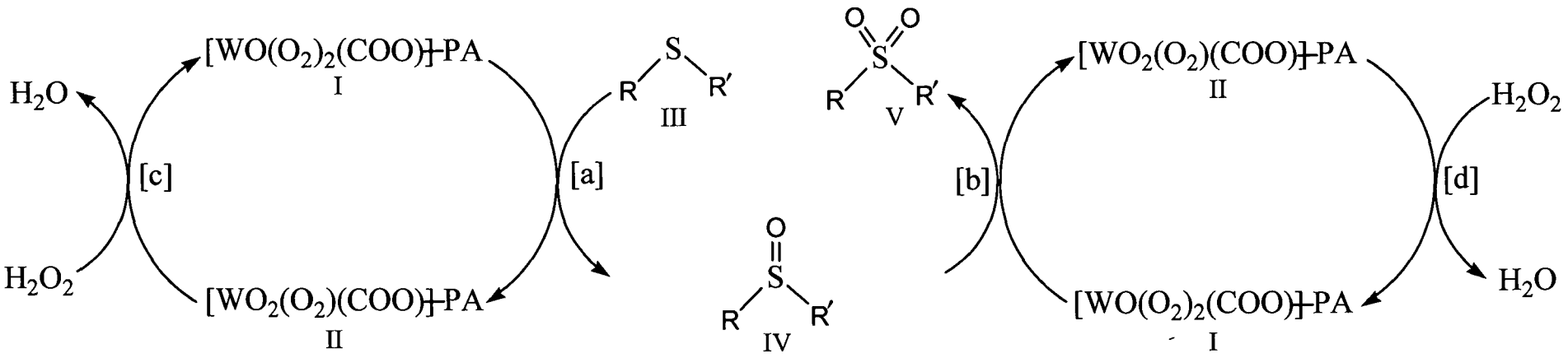


Fig 4.3. Schematic representation of reactions occurring with homogeneous catalysts shown with **PAW** (**3.2**) as representative. [a] Transfer of electrophilic oxygen from **I** to the substrate **III** takes place to yield sulfoxide **IV** with concomitant formation of inactive monoperoxotungstate intermediate **II**. [b] The sulfoxide **IV** may further react with another mole of peroxotungstate to form sulfone **V**. [c], [d] Intermediate **II** reacts with exogenous H_2O_2 to yield the reactive diperoxotungstate species **I** giving rise to a catalytic cycle. No attempt is made to show the exact stoichiometry of the reaction.

4.4 CONCLUSIONS

In summary, it has been demonstrated that the polymer immobilized pW complex **PANW (3.1)** could act as efficient heterogeneous catalyst in selective oxidation of organic sulfides by hydrogen peroxide under mild reaction conditions. The water soluble polymer anchored compounds on the other hand, serve as homogeneous catalysts in sulfide oxidations. It is remarkable that the catalysts, irrespective of being homogeneous or heterogeneous, can be effectively used for obtaining high purity sulfoxide as well as sulfone from the corresponding aryl or alkyl sulfides and DBT in excellent yield and high TOF by a versatile variation of reaction conditions. The heterogeneous catalyst displayed superior activity over its homogeneous counterparts. The significant advantages offered by the developed protocol include (i) control over degree of oxidation of products; (ii) chemoselectivity of the catalyst toward sulfur group of substituted sulfides and sulfoxides with other oxidation prone functional groups; (iii) avoidance of chlorinated solvent or other additives. Easy regeneration and reusability of the catalyst **PANW (3.1)** for several catalytic cycles without any change in its activity is an additional attractive feature associated with the catalyst. The findings confirm that immobilization of pW species on the PAN matrix enhances its stability, and leads to an improvement in the overall efficiency of the pW catalyst. Moreover, compounds can be easily prepared from readily available starting materials. It is reasonable to expect that the synthesized catalysts may emerge as useful additions to the range of ecologically suitable oxidation catalysts with the potential for accomplishing some of the goals of green chemistry.

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Appendix: 4A Characterization of Sulfoxides and Sulfones

(a) Methylphenylsulfoxide: Isolated as light yellow solid; mp 28-29°C; ν (KBr)/cm^{-1} 1047;

^{1}H NMR (400MHz; $CDCl_3$, δ): 2.73(s, 3H); 7.30-7.36(m, 1H); 7.40-7.49(m, 2H); 7.61-7.69(m, 2H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 43.92; 123.52; 128.68; 130.98; 145.49

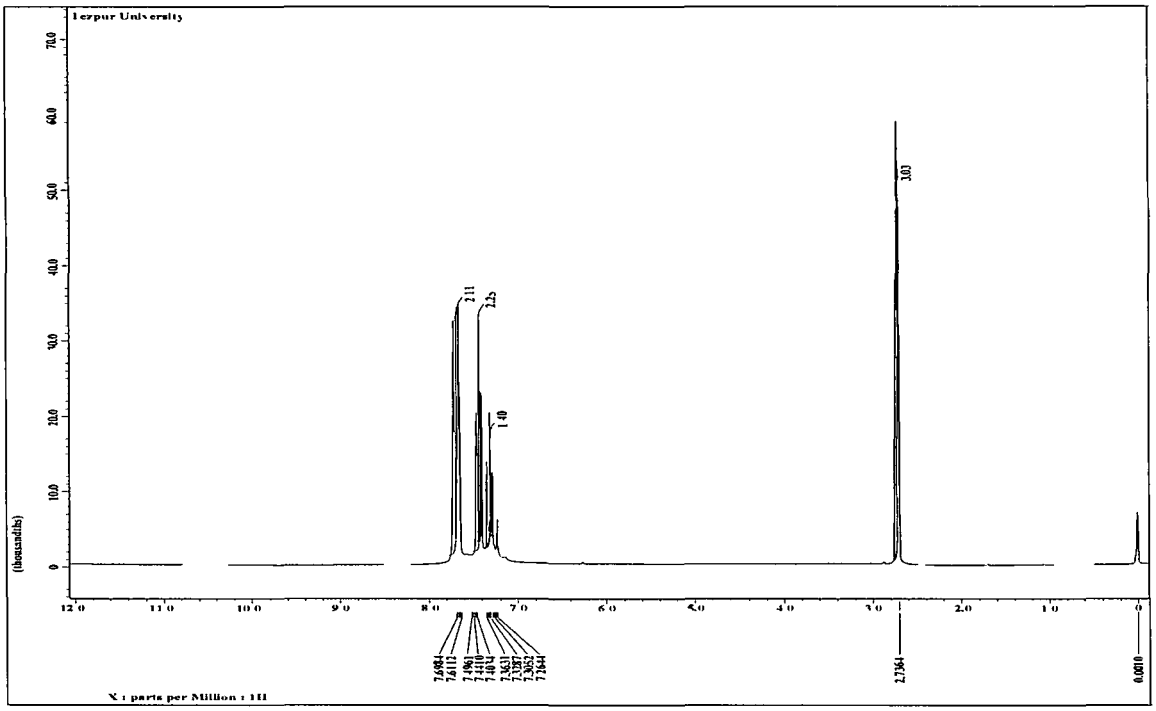


Fig. 4A.1 ^{1}H NMR spectra of methyl phenyl sulfoxide.

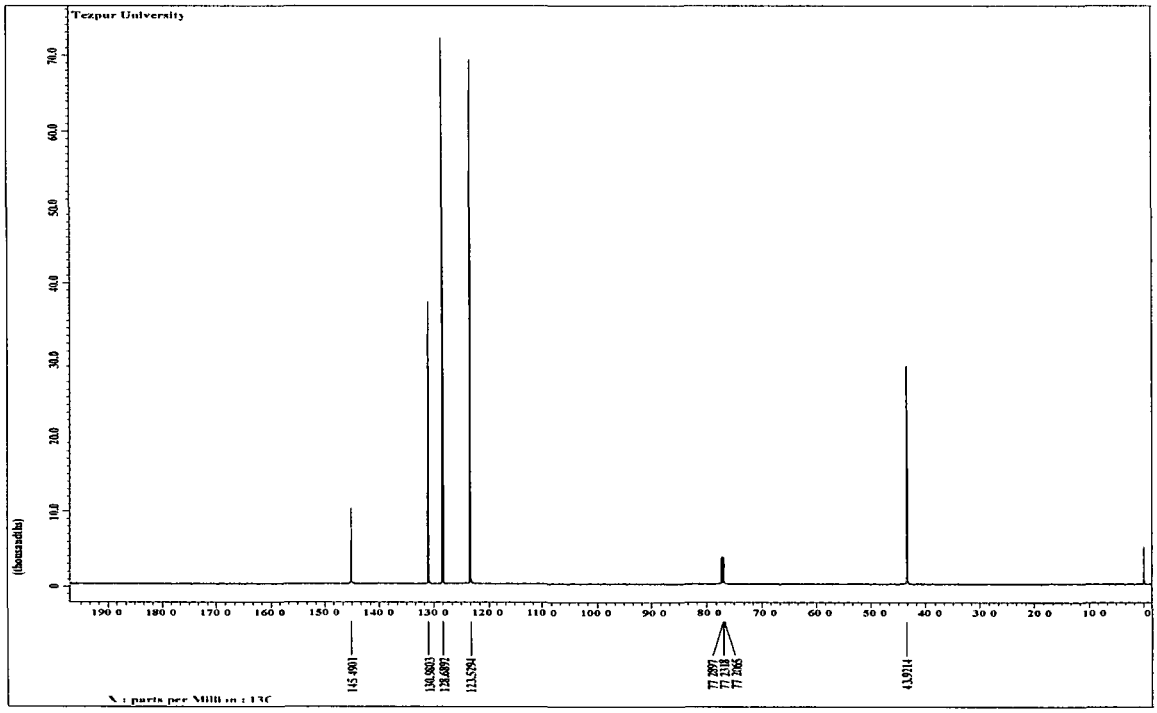


Fig. 4A.2 ^{13}C NMR spectra of methyl phenyl sulfoxide.

(b) Methylphenylsulfone: Isolated as white solid; mp 85-86°C; ν (KBr)/cm^{-1} 1320, 1164;

^{1}H NMR (400MHz; $CDCl_3$, δ): 3.01(s, 3H); 7.52-7.58(m, 1H); 7.61-7.69(m, 2H); 7.91-7.95(m, 2H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 44.82; 126.21; 128.52; 133.24; 137.42

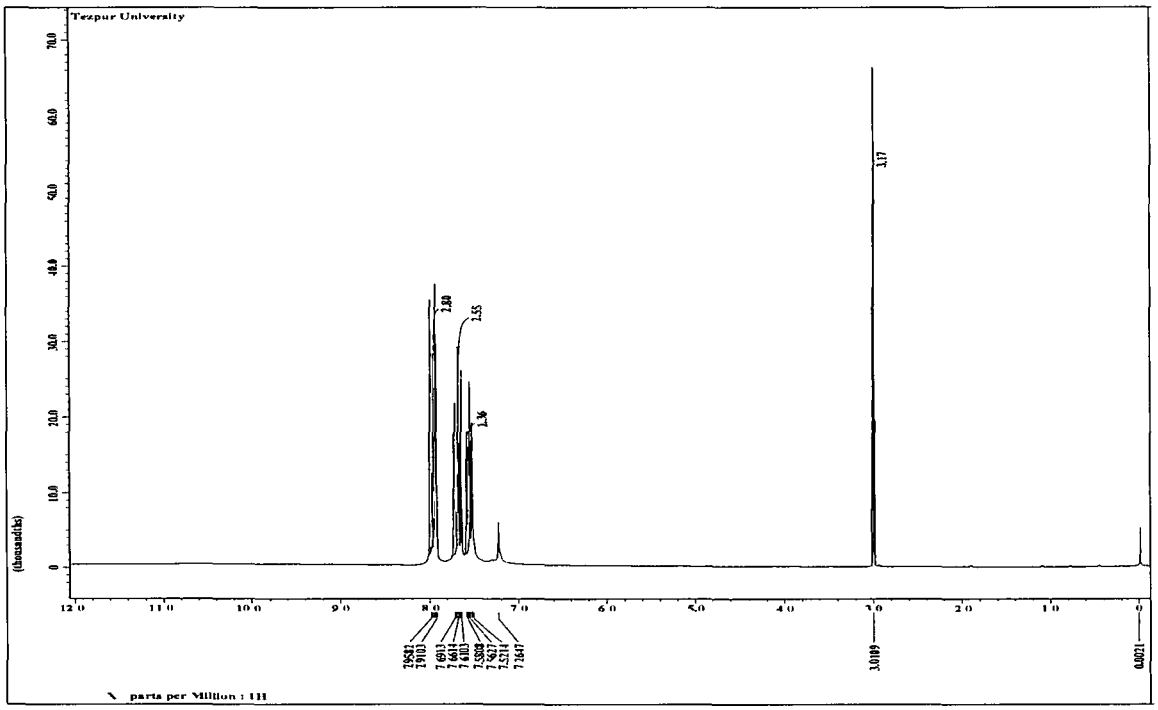


Fig. 4A.3 ^{1}H NMR spectra of methyl phenyl sulfone.

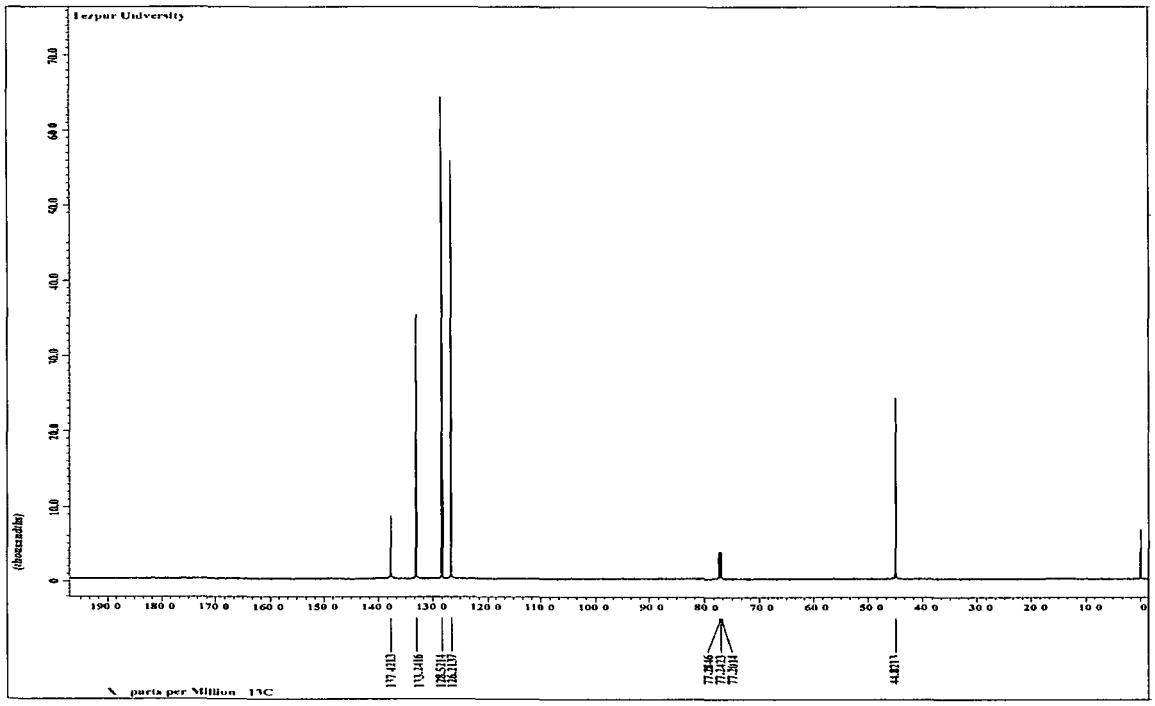


Fig. 4A.4 ^{13}C NMR spectra of methyl phenyl sulfone.

(c) Dimethylsulfoxide: Isolated as liquid; ν (KBr)/cm^{-1} 1050;

^1H NMR (400MHz; $CDCl_3$, δ): 2.61(s, 6H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 40.54

(d) Dimethylsulfone: Isolated as white solid; mp 236-237°C; ν (KBr)/cm^{-1} 1316, 1139;

^1H NMR (400MHz; $CDCl_3$, δ): 3.28(s, 6H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 44.61

(e) Dibutylsulfoxide: Isolated as white solid; mp 32-33°C; ν (KBr)/cm^{-1} 1061;

^1H NMR (400MHz; $CDCl_3$, δ): 0.96(t, 6H, J=7.41Hz); 1.37-1.48(m, 4H); 1.69-1.78(m, 4H); 2.62-2.68(m, 4H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 13.72; 22.15; 24.50; 51.96

(f) Dibutylsulfone: Isolated as white solid; mp 42-43°C; ν (KBr)/cm^{-1} 1344, 1134;

^1H NMR (400MHz; $CDCl_3$, δ): 0.96 (t, 6H, J=7.41Hz); 1.39-1.49 (m, 4H); 1.79-1.89(m, 4H); 2.87-2.93(m, 4H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 13.52; 21.76; 23.92; 52.54

(g) Butylpropylsulfoxide: Isolated as liquid; ν (KBr)/cm^{-1} 1059;

^1H NMR (400MHz; $CDCl_3$, δ): 0.93-0.99(m, 6H); 1.29-1.39(m, 2H); 1.70-1.79(m, 4H); 2.54-2.60(m, 4H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 13.72; 22.15; 24.50; 51.96

(h) Butylpropylsulfone: Isolated as white solid; mp 81-82°C; ν (KBr)/cm^{-1} 1341, 1133;

^1H NMR (400MHz; $CDCl_3$, δ): 0.94-0.99(m, 6H); 1.29-1.38(m, 2H); 1.81-1.89(m, 4H); 2.85-2.94(m, 4H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 13.52; 21.76; 23.92; 52.54

(i) Dibenzothiophene sulfoxide: Isolated as white solid; mp 188-189°C; ν (KBr)/cm^{-1} 1049;

^1H NMR (400MHz; $CDCl_3$, δ): 7.51-7.58(m, 2H); 7.70-7.74(m, 2H); 7.92-7.98(m, 4H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 123.47; 124.19; 126.39; 129.89; 132.65; 143.32

(j) Dibenzothiophene sulfone: Isolated as white solid; mp 231-232°C; ν (KBr)/cm^{-1} 1368, 1167;

^1H NMR (400MHz; $CDCl_3$, δ): 7.50-7.54(m, 2H); 7.61-7.65(m, 2H); 7.77-7.83(m, 4H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 121.56; 121.96; 130.19; 131.56; 133.72; 137.64

(k) Phenylvinylsulfoxide: Isolated as pale yellow liquid; ν (KBr)/cm^{-1} 1053;

^1H NMR (400MHz; $CDCl_3$, δ): 5.93(d, 1H, J=10.13Hz); 6.28(d, 1H, J=15.89Hz); 6.56-6.69(m, 1H); 7.27-7.36(m, 1H); 7.45-7.52(m, 2H); 7.63-7.69(m, 2H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 120.59; 124.59; 129.35; 131.17; 142.96; 143.40

(l) Phenylvinylsulfone: Isolated as white solid; mp 63-64°C; ν (KBr)/cm^{-1} 1365, 1162;

^1H NMR (400MHz; $CDCl_3$, δ): 6.12(d, 1H, J=9.60Hz); 6.44(d, 1H, J=16.42Hz); 6.64-6.77(m, 1H); 7.44-7.52(m, 1H); 7.58-7.67(m, 2H); 7.87-7.91(m, 2H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 127.85; 129.33; 133.77; 138.51; 139.70

(m) 2-(Phenylsulfinyl)ethanol: Isolated as light brown solid; mp 42-43°C; ν (KBr)/cm^{-1} 1039;

^{1}H NMR (400MHz; $CDCl_3$, δ): 2.41(s, 1H); 3.13(t, 2H, J=5.31Hz); 3.86(t, 2H, J=5.26Hz); 7.28-7.37(m, 1H); 7.48-7.56(m, 2H); 7.64-7.69(m, 2H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 56.19; 60.94; 125.41; 129.99; 131.24; 144.51

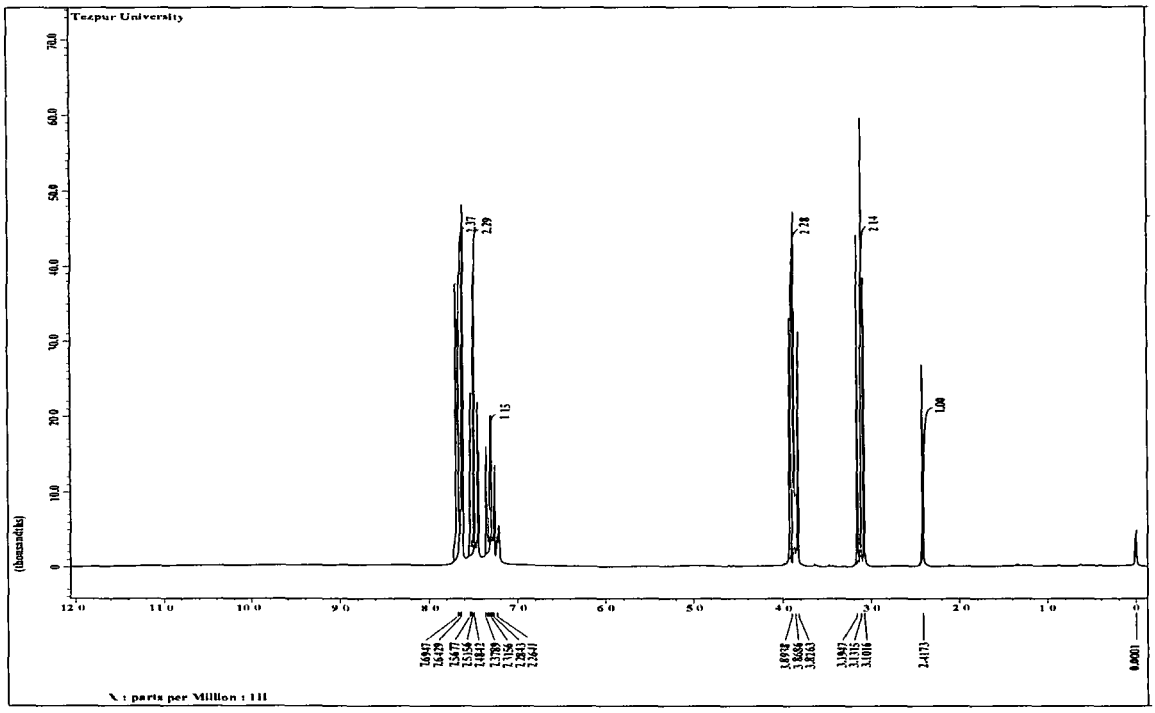


Fig. 4A.5 ^{1}H NMR spectra of 2-(Phenylsulfinyl)ethanol.

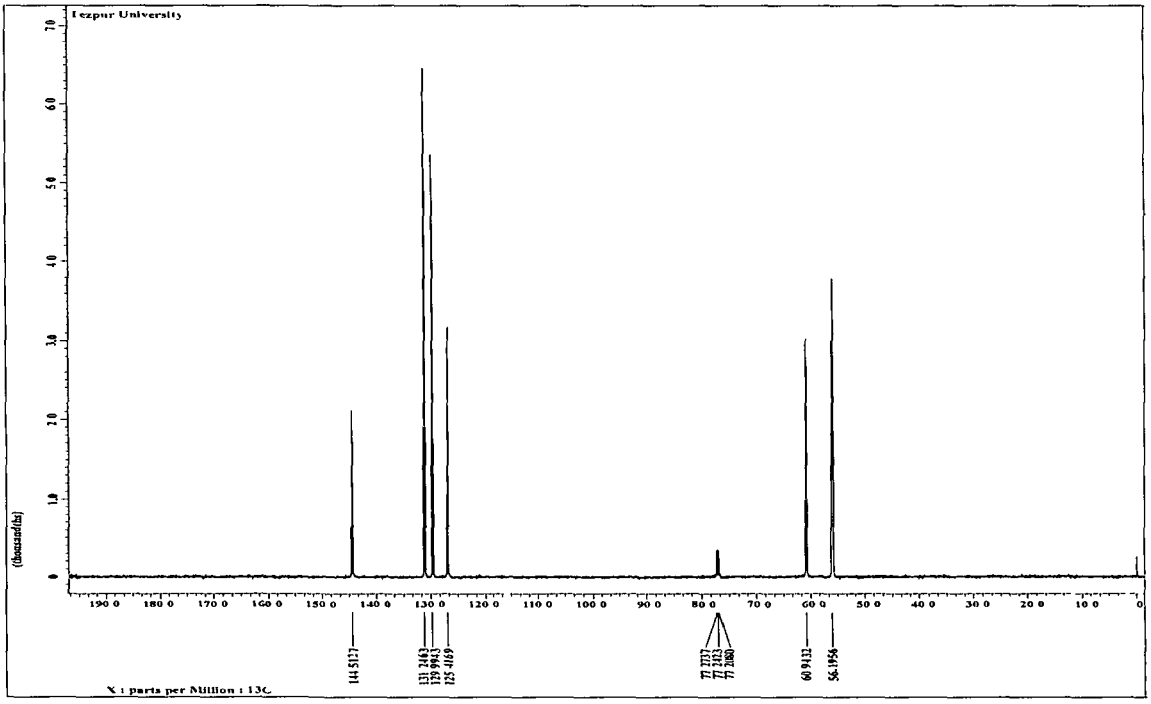


Fig. 4A.6 ^{13}C NMR spectra of 2-(Phenylsulfinyl)ethanol.

(n) 2-(Phenylsulfonyl)ethanol: Isolated as white solid; mp 96-97°C; ν (KBr)/cm^{-1} 1338, 1155;

^{1}H NMR (400MHz; $CDCl_3$, δ): 2.49(s, 1H); 3.31(t, 2H, J=5.46Hz); 3.96(t, 2H, J=5.26Hz); 7.45-7.52(m, 1H); 7.58-7.67(m, 2H); 7.89-7.96(m, 2H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 57.39; 61.57; 128.93; 129.54; 134.06; 140.74

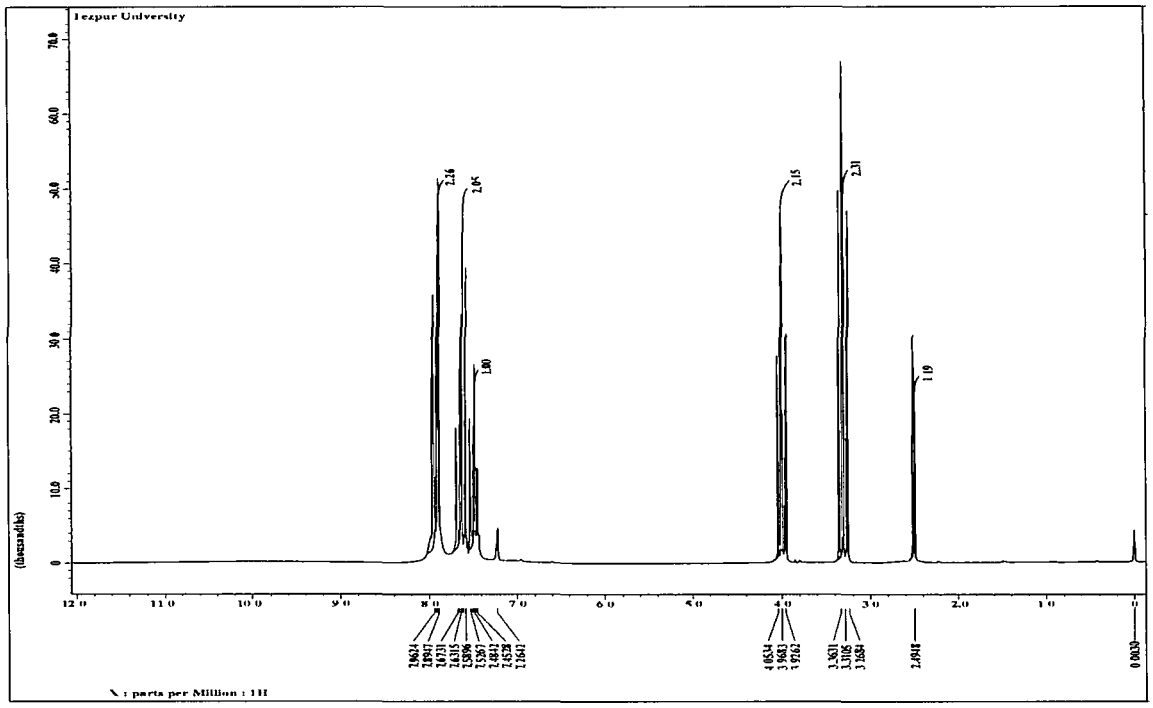


Fig. 4A.7 ^{1}H NMR spectra of 2-(Phenylsulfonyl)ethanol.

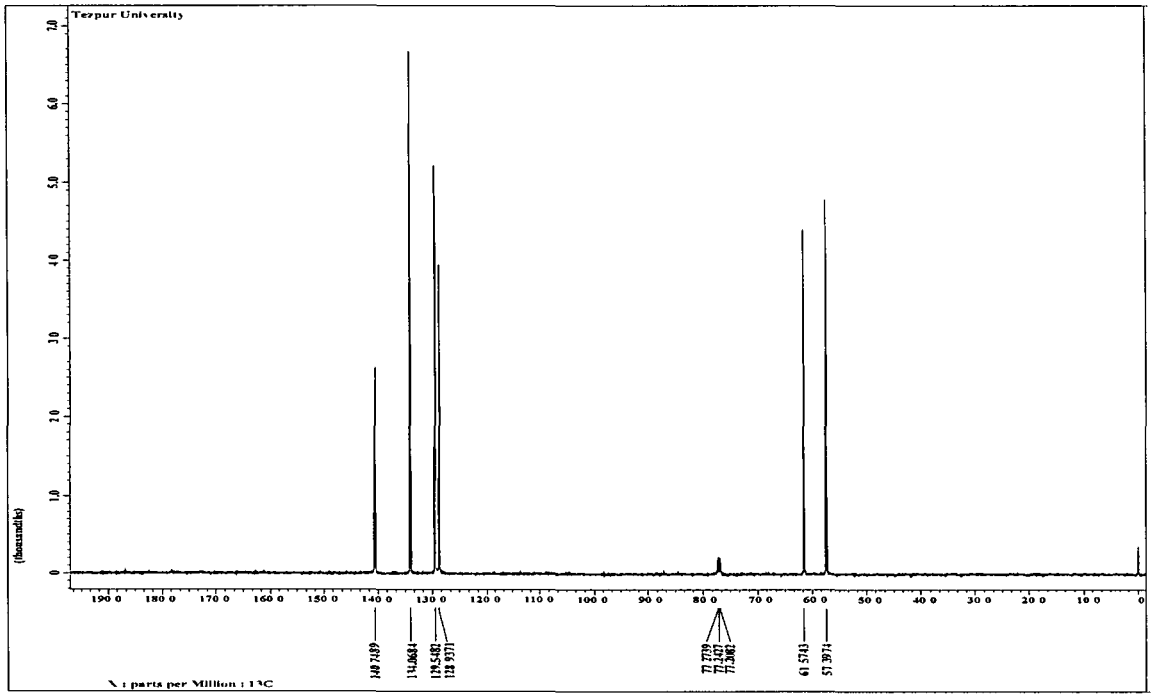


Fig. 4A.8 ^{13}C NMR spectra of 2-(Phenylsulfonyl)ethanol.

(o) Allylphenylsulfoxide: Isolated as pale yellow liquid; ν (KBr)/cm^{-1} 1044;

^{1}H NMR (400MHz; $CDCl_3$, δ): 3.41(dt, 2H, J=7.11, 1.12Hz); 5.00(dq, 1H, J=1.42, 17.10Hz); 5.15(dq, 1H, J=1.12, 10.22Hz); 5.45(ddt, 1H, J=7.11, 10.22, 17.10 Hz); 7.26-7.30(m, 1H); 7.37-7.39(m, 2H); 7.60-7.63(m, 2H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 60.38; 117.99; 124.70; 125.08; 129.09; 131.24; 142.19

(p) Allylphenylsulfone: Isolated as pale yellow liquid; ν (KBr)/cm^{-1} 1319, 1147;

^{1}H NMR (400MHz; $CDCl_3$, δ): 3.94(dt, 2H, J= 7.19, 1.22Hz); 5.00(dq, 1H, J= 1.48, 17.21Hz); 5.17(dq,1H, J= 1.22, 10.31Hz); 5.62(ddt, 1H, J= 7.19, 10.31, 17.21Hz); 7.38-7.41(m, 1H); 7.63-7.69(m, 2H); 7.90-7.93(m, 2H)

^{13}C NMR (100.5MHz; $CDCl_3$, δ): 60.66; 117.56; 124.66; 128.87; 129.07; 133.79; 138.25

Splitting patterns are designated as s (singlet), d (dublet), t (triplet), dt (double triplet), ddt (double-double triplet), q (quartet), dq (double quartet), m (multiplet).

CHAPTER 5

Safe and Selective Oxidation of Organic Sulfides by Mononuclear and Dinuclear Peroxotungsten(VI) Complexes

5.1 INTRODUCTION

The most straightforward method for preparing sulfoxides or sulfones, both of which are important commodity chemicals, involve oxidation of sulfides[1-3]. A myriad oxidizing agents are available for the purpose[4] which include nitric acid[5], $KMnO_4$[6], MnO_2[7], $NaClO_4$[8], *m*-chloroperbenzoic acid[9], sodium metaperiodate[10,11], bromine[12], nitrogen tetraoxide[13], oxaziridine[14], benzeneseleninic peracid[15,16], *tert*-butyl hydroperoxide[17], sulfinyl peroxy compounds[18], iodosobenzene diacetate[19] and 4-methylmorpholine *N*-oxide with osmium tetraoxide[20]. Unfortunately, most of these reagents are not satisfactory because factors such as low content of effective oxygen, formation of environmentally unfavourable by-products and poor selectivity, over oxidation and difficulty in regeneration of the reagent often limit their practical utility. In addition, the reactions may require many hours and high temperature. Hydrogen peroxide, although is a very attractive oxidant for liquid–phase reactions[21,22], it should be noted that H_2O_2 can be an ideal, waste-avoiding oxidant only when it is used in a controlled manner without hazardous organic solvents and other toxic compounds[23]. In view of the above, research in this area continues in the quest of newer reagents and protocols for selective oxidation under easy operational conditions.

The use of diperoxomolybdenum complexes, particularly the Mimoun complexes, as stoichiometric reagents as well as catalysts in oxygen transfer reactions is well established[24,25]. In contrast, reports on application of discreet peroxotungsten complexes, dinuclear pW complexes in particular, as organic oxidant is scanty[26-30]. The dimeric pW anions of the type $[W_2O_3(O_2)_4]^{2-}$ are believed to be the species responsible for tungstate catalyzed oxidations by H_2O_2[24,26,31].

It is pertinent to mention that a set of dinuclear peroxotungsten (pW) compounds with biogenic co-ligands of the type, **$Na_2[W_2O_3(O_2)_4(L)_2].3H_2O$, L = glycyl-glycine (DWG) (5.2)**[26] and glycyl-leucine,[26] and **$Na_2[W_2O_3(O_2)_4$(cystine)$].4H_2O$ (DWC) (5.3)**[32] and their monomeric pW analogues, **$[WO(O_2)_2(L)(H_2O)].3H_2O$, L = glycyl-glycine (MWG) (5.1)**[33] and glycyl-leucine[33], reported from our laboratory was found to mediate bromination of organic substrates in aqueous organic media, with good activity at neutral pH and room temperature[26].

Inspired by these observation and as a sequel to our endeavour in developing newer reagents and methodologies for oxidations under environmentally acceptable reaction conditions, for the present work, our main concern has been to evaluate the efficacy of the anionic dinuclear as well as neutral monomeric pW complexes as selective and recoverable reagents for oxidation of thioethers and DBT to the corresponding sulfoxide or sulfone under mild conditions. Results of our investigation are reported herein. Comparisons of the two types of pW compounds are made and role of different reaction conditions in the selectivity are highlighted.

5.2 EXPERIMENTAL SECTION

5.2.1 Synthesis of $[WO(O_2)_2$(glycyl-glycine) $(H_2O)].3H_2O$ (MGW) (5.1)

The compound **MWG (5.1)** was synthesized according to a previously reported procedure[33]. This consisted of gradual addition of 5 mL H_2O_2 (30% solution, 44 mmol) to a mixture of H_2WO_4 (0.5 g, 1.5 mmol) and peptides at a molar ratio of W: dipeptide of 1:1 with continuous stirring. Keeping the temperature below 4 °C in an ice bath, the mixture was stirred for *ca.* 5 min until all solids dissolved. At this stage the pH of the solution was recorded to be *ca.* 1.5. The pH of the reaction mixture was raised up to 5.5 by adding NaOH solution (0.1M) dropwise. On adding pre-cooled acetone (about 50 mL) to the above solution under continuous stirring a colorless pasty mass separated out. After allowing to stand for about 10 min in the ice bath, the supernatant liquid was decanted off and the residue was treated repeatedly with distilled acetone under scratching until it became a white microcrystalline solid. The product was separated by centrifugation, washed with cold distilled acetone and dried in vacuo over concentrated sulfuric acid.

5.2.2 Synthesis of $Na_2[W_2O_3(O_2)_4$(glycyl-glycine)$_2]$. $3H_2O$ (DWG) (5.2)

The compound **DWG (5.2)** was prepared according to a previously reported procedure[26]. In a typical reaction, H_2WO_4 (0.25 g, 1.0 mmol) was added to 10 mL of 30% H_2O_2 (88.2 mmol) contained in a 250 mL beaker. The mixture was kept in an ice bath and stirred slowly until all the solids dissolved and a clear solution was obtained. To this solution dipeptide (1.0 mmol) was added. The pH of the clear solution was recorded to be 1.6. The pH of the reaction mixture was adjusted to *ca.* 2.5 by adding sodium

hydroxide (46.0 mmol). Subsequently, the compound was isolated by adding pre-cooled acetone (*ca.* 50 mL) as mentioned above.

5.2.3 Synthesis of $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ (DWC) (5.3)

The compound **DWC** (**5.3**) was synthesized according to the procedure reported earlier[32]. In a typical reaction, A_2WO_4 (0.5 g, 1.5mM) was added to a solution of cysteine (0.24 g, 1.5mM) in 5 mL of water. Keeping this mixture in an ice bath 5 mL of 30% H_2O_2 (44.1 mM) was added gradually with constant stirring until all solid dissolved and a clear colourless solution was obtained. The pH of the solution was recorded to be 2.5. No attempt was made to adjust the pH of the reaction solution. Subsequently, the compound was isolated by adding pre-cooled acetone (*ca.* 50 mL) as mentioned above.

5.2.4 General procedure for selective oxidation of sulfides to sulfoxides

To a stirred solution of sulfide (2.5 mmol) in CH_3OH/H_2O (1:1, 10 mL), the complex **MWG** (1.17 g, 2.5 mmol) or **DWC** (1.12 g, 1.25 mmol) or **DWG** (1.13 g, 1.25 mmol), were added successively maintaining molar ratio of W:substrate at 1:1, in a 50 mL two-necked round-bottomed flask. The resulting reaction mixture was stirred at room temperature. The progress of the reaction was monitored by thin layer chromatography (TLC) and GC. After completion of the reaction, the sulfoxide obtained was isolated, purified and characterized by methods similar to those mentioned under Section 4.2.1 of Chapter 4.

5.2.5 General procedure for selective oxidation of sulfides to sulfones

To a stirred solution of sulfide (1.25 mmol) in CH_3OH/H_2O (1:1, 10 mL), the complex **MWG** (1.17 g, 2.5 mmol) or **DWC** (1.12 g, 1.25 mmol) or **DWG** (1.13 g, 1.25 mmol) were added successively maintaining molar ratio of W:substrate at 2:1. After completion of the reaction, the sulfone obtained was isolated, purified and characterized by methods similar to those described under Section 4.2.1 of Chapter 4.

5.2.6 Regeneration of reagent

The regeneration of the compounds, **5.1-5.3** for reuse was tested for the reaction using methyl phenyl sulfide (MPS) as substrate. After extraction of the organic reaction product as mentioned under Section 5.2.4 and 5.2.5, the aqueous part of the reaction mixture was transferred to a 250 mL beaker. Keeping the solution in an ice bath, 30% H_2O_2 was added to it maintaining the W:peroxide ratio at 1:1, followed by addition of pre-cooled acetone with constant stirring until a white pasty mass separated out. The product was separated by centrifugation, washed with cold acetone and dried in vacuo over concentrated sulfuric acid. The regenerated pW compound was then placed into a fresh reaction mixture. In an alternative procedure, regeneration of the used reagent could be achieved by charging the spent reaction mixture, remaining after extraction of the organic reaction product, with 30% H_2O_2 maintaining the W:peroxide molar ratio at 1:1 and then repeating the experiment. The reagents were reused for up to seven reaction cycles by adding fresh lot of substrate and the required solvent after each cycle of reaction.

5.3 RESULTS AND DISCUSSION

The mononuclear as well as dinuclear diperoxo complexes of tungsten of the type, $[WO(O_2)_2(glycyl\text{-}glycine)(H_2O)].3H_2O$ (**MWG**) (**5.1**), $Na_2[W_2O_3(O_2)_4(glycyl\text{-}glycine)_2].3H_2O$ (**DWG**) (**5.2**) or $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ (**DWC**) (**5.3**), used as stoichiometric oxidants of sulfide in the present study (Fig. 5.1), are water soluble and stable in aqueous solution in a wide range of pH values[26,32]. The compounds were prepared by reaction of H_2WO_4 with 30% H_2O_2 and the respective ligand in aqueous medium, by methods previously reported from our laboratory[26,32].

5.3.1 Activity of MWG (5.1), DWG (5.2) and DWC (5.3) as sulfide oxidant

The compounds, **5.1-5.3** were evaluated for their oxidation of a variety of aryl and alkyl sulfides as well as dibenzothiophene (DBT). Based on the results of trial runs, the reaction conditions were optimized by using methyl phenyl sulfide (MPS) as the model substrate and **MWG (5.1)** as the oxidant, as presented in Table 5.1.

5.3.1.1 Effect of solvent

The reaction of the substrate with the complex in the molar ratio of 1:1 in CH_3OH/H_2O (1:1) proceeded smoothly at ambient temperature to selectively afford sulfoxide in impressive yields within less than an hour. No overoxidation to sulfone was noted even on prolonging the reaction time. The results are summarized in Table 5.2. Interestingly, no oxidation was observed in presence of pure organic solvents used (Table 5.1, entries 7-12). When water was included in the water miscible solvent system, facile transformation of MPS to the desired product was achieved (Table 5.1, entries 1-3).

This lack of reactivity in pure organic solvents is apparently due to the insolubility of the oxidant in the neat organic solvents. The observation is in contrast to the catalytic oxidation of the sulfides with H_2O_2 in presence of the polymer anchored complexes **PANW (3.1)**, **PAW (3.2)**, **PMAW (3.3)** and **PAmW (3.4)** which proceeded smoothly in presence of neat methanol or acetonitrile used as solvent, probably because the required water in these reactions is provided by the oxidant, aqueous H_2O_2. Evidently, solvation of the complexes is a pre-requisite for their oxidant activity. It is plausible that water molecule would co-ordinate to the W(VI) centre of the intermediate complexes, formed in solution during the oxidation reaction, thereby stabilizing such species[23,24].

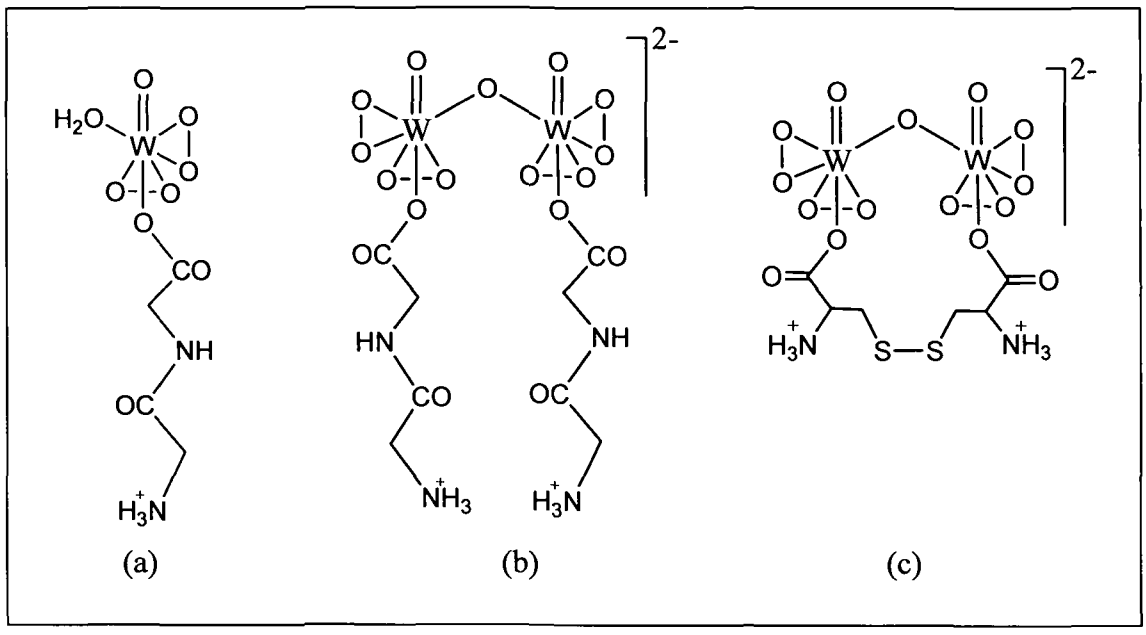
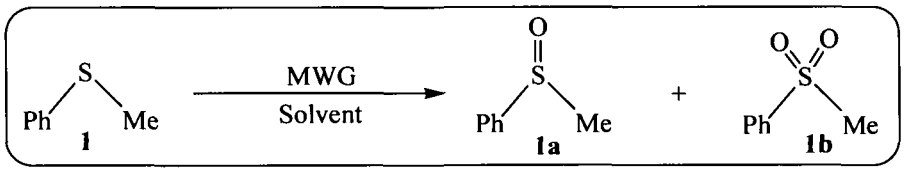


Fig. 5.1 Complexes used as stoichiometric oxidants of sulfides in the current study, (a) **MWG (5.1)**, (b) **DWG (5.2)** and (c) **DWC (5.3)**

Table 5.1 Optimization of reaction conditions for the selective oxidation of methyl phenyl sulfide (MPS) to sulfoxide and sulfone by **MWG**[a]

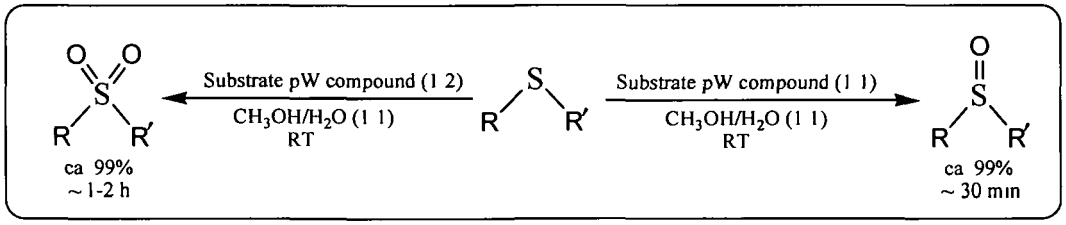


Entry	Molar ratio (MPS:**MWG**)	Solvent	Time (h)	Isolated Yield (%)	
				1a	**1b**
1	1:1	CH_3OH:H_2O (1:1)	0.50	99	0
2	1:1	EtOH:H_2O (1:1)	0.58	98	0
3	1:1	CH_3CN:H_2O (1:1)	0.67	99	0
4[b]	1:2	CH_3OH:H_2O (1:1)	0.75	69	27
5[b]	1:2	CH_3OH:H_2O (1:1)	1.16	0	99
6	2:1	CH_3OH:H_2O (1:1)	0.58	49	0
7	1:1	CH_3OH	6.00	No reaction	
8	1:1	EtOH	6.00	No reaction	
9	1:1	CH_3CN	6.00	No reaction	
10	1:1	DCM	6.00	No reaction	
11	1:1	CH_3Cl	6.00	No reaction	

[a]All reactions were carried out with 2.5 mmol of MPS in 10 mL of solvent at RT, unless otherwise indicated.
[b]Reaction conditions: **MWG** (1.17 g, 2.50 mmol), MPS (1.25 mmol), CH_3OH/H_2O (1:1).

5.3.1.2 Effect of substrate : oxidant ratio

The substrate:oxidant stoichiometry was found to play a crucial role in determining the activity and selectivity of the compounds in the oxidation reaction (Scheme 5.1). The reactions were examined under varying substrate:oxidant molar ratios under the standard reaction conditions (Table 5.1, entries 3-6). It is noteworthy that whereas a 1:1 molar ratio of substrate:compound afforded sulfoxide, clean conversion to sulfone could be achieved for each of the substrates tested (Table 5.3), in excellent yield and at room temperature under analogous reaction conditions, simply by changing the substrate:compound molar ratio to 1:2. On further altering the substrate:oxidant stoichiometry to 2:1 sulfoxide was obtained as the exclusive product although, in this case only *ca.* 50% conversion was noted. The procedure thus offers control over degree of oxidation of the products (Scheme 5.1). Similar trend was observed in case of the dimeric compounds, **DWG (5.2)** and **DWC (5.3)** with diperoxotungsten moieties (Table 5.2 and 5.3). The oxidant activity of the two dimeric compounds were found to be comparable. The dimeric compounds however, exhibited slightly superior activity compared to the monomeric analogue **MWG (5.1)** in terms of reaction time.



Scheme 5.1

5.3.1.3 Effect of temperature

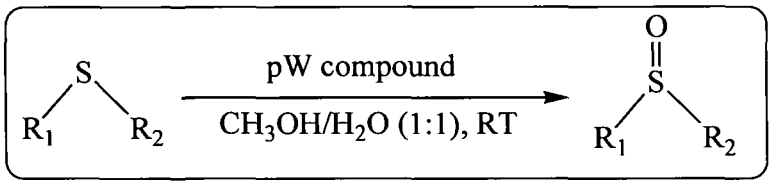
The reaction temperature was found to have a marked effect on the rate of the reaction. We have carried out the reactions at three different reaction temperatures viz., room temperature (RT), 50 °C and 65 °C. The data included in Table 5.2 and 5.3 show that by increasing the temperature, reaction time could be reduced substantially without affecting the selectivity.

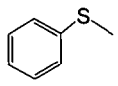
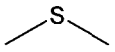
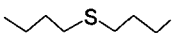
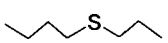
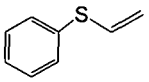
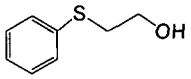
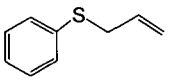
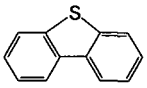
5.3.2 Selective oxidation of sulfides to sulfoxides or sulfones using MWG (5.1), DWG (5.2) or DWC (5.3) as oxidant

Apart from MPS, a range of organic sulfides underwent clean and selective oxidation to the corresponding sulfoxide under the optimum condition in presence of each of the complexes (Table 5.2). The data presented in Table 5.3 shows that the protocol also work efficiently in oxidizing sulfides to sulfones. The reagents exhibited complete chemoselectivity toward sulfur group of substituted sulfides containing other vulnerable groups such as –C=C– and –OH (Table 5.2 and 5.3, entries 5, 6 and 7).

Most importantly, dibenzothiophene (DBT) was selectively oxidized with reasonably good success at 65 °C (Table 5.2 and 5.3, entry 8). As has been observed in the case of supported pW catalyzed DBT oxidation by H_2O_2 described in the previous Chapter, the oxidation of DBT by the free pW compounds was found to be rather slow at room temperature. Accordingly, the reaction was carried out at a higher temperature.

Table 5.2 Selective oxidation of sulfides to sulfoxides by **MWG, DWG** or **DWC**[a]



Entry	Substrate	S:**MWG** = 1:1[b]		S:**DWG** = 2:1[c]		S:**DWC** = 2:1[c]	
		Time (h)	Isolated Yield (%) Sulfoxide	Time (h)	Isolated Yield (%) Sulfoxide	Time (h)	Isolated Yield (%) Sulfoxide
1		0.50	99	0.36	98	0.41	99
		0.25[d]	98	0.16[d]	97	0.16[d]	98
		0.50	97[e]	0.36	95[e]	0.41	96[e]
		0.25[d]	96[e]	0.16[d]	93[e]	0.16[d]	95[e]
2		0.50	99	0.33	99	0.33	99
3		0.50	99	0.36	96	0.41	98
		0.25[d]	98	0.16[d]	99	0.16[d]	99
4		0.50	97	0.36	96	0.41	99
		0.25[d]	98	0.16[d]	99	0.16[d]	98
5		2.00	98	1.41	97	1.50	98
		1.00[d]	99	0.58[d]	98	0.66[d]	98
6		1.00	97	0.58	98	0.66	97
		0.50[d]	98	0.25[d]	99	0.33[d]	98
7		0.50	98	0.41	98	0.41	97
		0.25[d]	97	0.16[d]	96	0.16[d]	98
8		4.50[d]	97	3.66[d]	98	4.00[d]	98

[a]All reactions were carried out with 2.5 mmol of substrate in 10 mL of CH_3OH/H_2O (1:1) at RT, unless otherwise indicated.

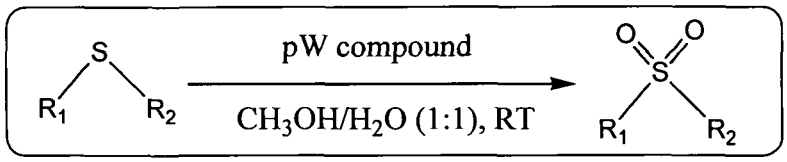
[b]Reaction condition: **MWG** (1.17 g, 2.50 mmol).

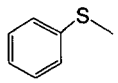
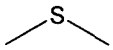
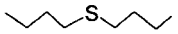
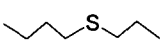
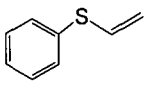
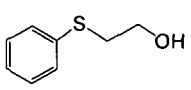
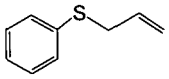
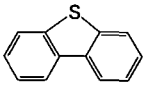
[c]Reaction conditions: **DWG** (1.13 g, 1.25 mmol) or **DWC** (1.12 g, 1.25 mmol).

[d]Reaction at 65 °C.

[e]Yield of 7th reaction cycle.

Table 5.3 Selective oxidation of sulfides to sulfones by **MWG, DWG** or **DWC**[a]



Entry	Substrate	S:**MWG** = 1:2[b]		S:**DWG** = 1:1[c]		S:**DWC** = 1:1[c]	
		Time (h)	Isolated Yield (%) Sulfone	Time (h)	Isolated Yield (%) Sulfone	Time (h)	Isolated Yield (%) Sulfone
1		1.16	99	0.91	98	1.00	99
		0.50[d]	98	0.25[d]	99	0.33[d]	99
		1.16	97[e]	0.91	95[e]	1.00	95[e]
		0.50[d]	94[e]	0.25[d]	96[e]	0.33[d]	97[e]
2		1.16	99	0.83	98	0.83	99
3		1.16	98	0.91	97	1.00	98
		0.50[d]	99	0.25[d]	98	0.33[d]	99
4		1.16	98	0.91	98	1.00	97
		0.50[d]	98	0.25[d]	99	0.33[d]	98
5		4.00	99	2.91	96	3.00	97
		2.00[d]	98	1.16[d]	99	1.25[d]	98
6		2.00	97	1.41	96	1.50	98
		1.00[d]	98	0.66[d]	98	0.75[d]	99
7		1.16	99	1.00	97	1.00	99
		0.50[d]	98	0.33[d]	99	0.33[d]	98
8		8.00[d,f]	98	6.66[d,g]	96	7.00[d,g]	97

[a]All reactions were carried out with 1.25 mmol of substrate in 10 mL of CH_3OH/H_2O (1:1) at RT, unless otherwise indicated.

[b]Reaction condition: **MWG** (1.17 g, 2.50 mmol).

[c]Reaction conditions: **DWG** (1.13 g, 1.25 mmol) or **DWC** (1.12 g, 1.25 mmol).

[d]Reaction at 65 °C.

[e]Yield of 7th reaction cycle.

[f]Conditions: molar ratio of **MWG**:substrate = 2.5:1.

[g]Conditions: molar ratio of **DWG**:substrate or **DWC**:substrate = 1.25:1.

5.3.3 Regeneration of the oxidant

Addition of H_2O_2 at the end of the reaction fully restores the oxidizing ability of each of the oxidants used, irrespective of it being monomeric or dimeric. The regeneration and recyclability of the complexes were assessed by using MPS as the model substrate. This was accomplished by charging the spent reaction mixture with 30% H_2O_2 by maintaining the molar ratio of W:H_2O_2 at 1:1, after separating the oxidized product and unreacted sulfide from the reaction mixture by extraction with ether. The reagents were reused for upto seven reaction cycles by adding fresh lot of H_2O_2, substrate and the required solvent after each cycle of reaction. The data presented in Table 5.2 [entry 1[e]] and Table 5.3 [entry 1[e]] shows that the oxidants remain effective with consistent activity and selectivity even after seventh cycle of reaction. The findings further indicate that the compounds are stable and are capable of retaining their structural integrity even after several reaction cycles.

It was of significance to note that the oxidant activity of each of the dimeric tetraperoxo complexes with two oxo-bridged diperoxotungsten units as well as the monomeric diperoxotungsten complex was recorded to be limited to *ca.* 50% of that expected on the basis of the total number of peroxo groups present in the complexes (Table 5.1, entry 6). Similar observation was made earlier with respect to the activity of the compounds in oxidative bromination of organic compounds[26]. This consistent observation cause us to infer that only one of the peroxo groups of a diperoxotungsten moiety present in each of the compounds, irrespective of being monomeric or dimeric, would be active in sulfide oxidation. Formation of an inactive monoperoxo intermediate from a catalytically active diperoxotungsten or molybdenum complex during oxygen-

atom transfer reactions mediated by such complexes has also been observed and reported previously by others[34,35].

5.3.4 Nature of the reaction intermediate

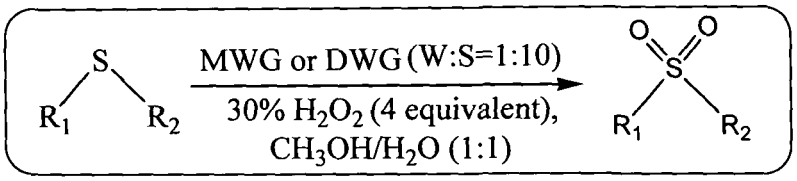
In order to rationalize the reaction sequence it was necessary to ascertain the nature of the tungsten species, formed in solution. Therefore, the product isolated from the aqueous extract of the spent reaction mixture, from experiments conducted separately using each of the complexes **MWG (5.1)**, **DWG (5.2)** or **DWC (5.3)** as oxidant, was subjected to spectral and elemental analysis as mentioned in our earlier paper[26]. Elemental analysis results suggested a 1:1 ratio for W:peroxo indicating the presence of monoperoxo tungsten species in each case. IR spectrum of each of the compounds, resembled closely the corresponding spectrum of the original starting complex showing the presence of peroxo group, terminal oxo groups, dipeptide ligand and co-ordinated water. In addition, the spectra of the intermediate complexes derived from **DWG (5.2)** and **DWC (5.3)** evidenced for the presence of μ-oxotungsten(VI) moiety. It thus appears that the dimeric complexes remain intact throughout the course of the oxidation process by retaining the W-O-W unit. Based on these observations the tungsten complex formed during the oxidation reaction was formulated as $[WO_2(O_2)L(H_2O)]$ (formed from monomeric diperoxo **MWG**) or a dimeric oxobridged complex of the type, $Na_2\{[WO_2(O_2)L(H_2O)]_2O\}$(originating from **DWG** or **DWC**).

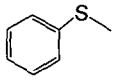
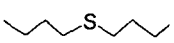
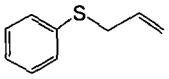
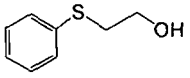
5.3.5 Peroxotungsten compounds as catalysts for oxidation of sulfides with H_2O_2

We have further explored catalytic behavior of the pW complexes in the oxidation of organic sulfides by using H_2O_2 as the co-oxidant, under mild reaction conditions. The

conditions of reaction were optimized by using MPS as the suitable substrate and **MWG (5.1)** as the representative catalyst. The reaction of MPS with 30% H_2O_2 (4 equivalents) in presence of the catalyst (W:substrate molar ratio of 1:100) in methanol/H_2O proceeded smoothly to afford a mixture of sulfoxide and sulfone in the ratio of 33:67 with a turn over frequency (TOF) of 26 h^{-1}. The reactions were conducted at room temperature under magnetic stirring. Our attempts to control the degree of oxidation by maintaining a lower oxidant or catalyst concentration, in order to attain selectivity with respect to sulfoxide, were unsuccessful. Interestingly however, selective oxidation of MPS to the corresponding sulfone could be achieved by increasing the amount of catalyst (10 fold). A W:substrate molar ratio of 1:10 was thus found to be optimum for the complete conversion of MPS into sulfone under the reaction conditions used. In a similar way dibutyl sulfide, allylphenyl sulfide and 2-(phenylthio)ethanol furnished the corresponding sulfone in high yields (Table 5.4). Most importantly, the TOF of the conversions could be substantially increased by carrying out the reactions under reflux at 65 °C. The co-existing alcohol and olefinic moieties in substituted sulfides remained unaffected. The results presented in Table 5.4 (entries 2, 3 and 4) show that the oxidation of sulfides to the corresponding sulfones has been achieved with complete chemoselectivity. The catalysts afforded regeneration *in situ* and could be reused without further conditioning after separating the oxidized product and unreacted sulfide from the reaction mixture by extraction with ether. The catalyst was reused for upto seven catalytic cycles with consistent activity and selectivity (Table 5.4, entry 1^f).

Table 5.4 Selective oxidation of sulfides to sulfones with 30% H_2O_2 catalyzed by mononuclear and dinuclear pW compound[a]



Entry	Substrate	MWG[b]			DWG[c]		
		Time (h)	Isolated Yield (%)	TOF[e] (h^{-1})	Time (h)	Isolated Yield (%)	TOF[e] (h^{-1})
1		1.00	98	10	0.75	99	13
		0.33[d]	99	30	0.20[d]	97	48
		1.00	96[f]	9	0.75	98[f]	13
		0.33[d]	98[f]	29	0.20[d]	96[f]	48
2		0.91	97	11	0.66	96	14
		0.30[d]	96	32	0.16[d]	98	61
3		1.08	95	9	0.83	97	12
		0.41[d]	93	22	0.25[d]	95	38
4		1.83	98	5	1.33	96	7
		0.75[d]	97	13	0.50[d]	98	19

[a]All reactions were carried out with 2.5 mmol of substrate, 4 equivalent of 30% H_2O_2 (with respect to substrate), 7 mL of CH_3OH/H_2O (1:1) at RT, unless otherwise indicated.

[b]Reaction condition: **MWG** (0.117 g, 0.250 mmol).

[c]Reaction conditions: **DWG** (0.113 g, 0.125 mmol) or **DWC** (0.112 g, 0.125 mmol).

[d]Reaction at 65 °C.

[e]TOF (Turnover frequency) = mmol of product per mmol of catalyst per hour (calculated on the basis of W content.)

[f]Yield of 7th reaction cycle.

5.3.6 The Proposed Mechanism

A possible mechanism consistent with the observations made in the present study is shown in Fig. 5.2, using **MWG (5.1)** as a representative oxidant. The diperoxotungsten(VI) compound **I** oxidizes substrate **III** to sulfoxide **IV** by transferring its electrophilic oxygen with the simultaneous formation of the monoperoxotungsten intermediate **II** (reaction a), as has been proposed in case of polymeric diperoxotungsten complexes (vide Chapter 4). The sulfoxide may subsequently react with another mole of peroxotungstate to form sulfone **V** (reaction b). In the presence of exogeneous H_2O_2 the monoperoxoW(VI) intermediate **II** combines with peroxide to regenerate the starting diperoxotungstate complex **I** giving rise to a catalytic cycle (reactions c and d). The reaction involving the compounds **DWG (5.2)** or **DWC (5.3)** with diperoxotungsten units, $\{[WO(O_2)_2L]_2O\}^{2-}$ (L = glycyl-glycine or cystine) is likely to proceed in a similar way via the formation of the oxo-bridged intermediate of the type $\{[WO_2(O_2)L]_2O\}^{2-}$ with two monoperoxo-W(VI) moieties. The observed reaction pattern is in accord with the earlier suggestions that for a peroxotungsten complex to be active in oxidation, an oxo-diperoxo configuration may be a prerequisite[26,35,36].

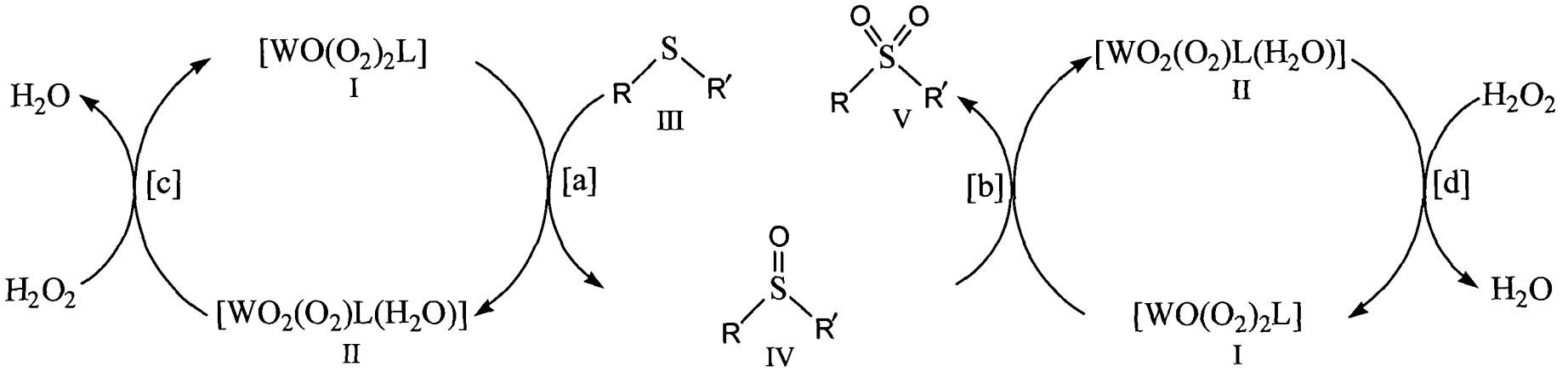


Fig. 5.2. Schematic representation of reactions occurring with **MWG** (L = glycyl-glycine). [a] Transfer of electrophilic oxygen from **I** to the substrate **III** takes place to yield sulfoxide **IV** with concomitant formation of inactive monoperoxotungstate intermediate **II**. [b] The sulfoxide **IV** may further react with another mole of peroxotungstate to form sulfone **V**. [c], [d] Intermediate **II** reacts with exogenous H_2O_2 to yield the reactive diperoxotungstate species **I** giving rise to a catalytic cycle. No attempt is made to show the exact stoichiometry of the reaction.

5.4 CONCLUSIONS

In conclusion, an efficient method for the selective oxidation of a variety of structurally diverse sulfides to sulfoxides, under environmentally clean conditions, has been developed using monomeric diperoxotungsten complex, **MWG (5.1)** as well as anionic dinuclear tetraperoxo tungsten complexes, **DWG (5.2)** and **DWC (5.3)** as stoichiometric oxidants. The attractive attributes of the methodology are: (i) it can be used for selectively obtaining sulfoxide or sulfone by a variation of reagent:substrate stoichiometry; (ii) chemoselectivity of the complexes toward the sulfur group of sulfides with co-existing oxidation prone functional groups; (iii) compounds can be stoichiometrically recovered in the presence of H_2O_2. Application to the refractory sulfur such as DBT make the procedure more generalized. Significantly, the compounds could also efficiently catalyze the selective oxidation of sulfides by H_2O_2 to yield sulfone with reasonably good TOF, under mild reaction conditions. The simplicity in the method of preparation of the reagents and redundancy of chlorinated solvents are additional advantages offered by the procedure. The developed methodologies thus conform to several guiding principles of green chemistry.

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CHAPTER 6

Polymer Bound Peroxotungsten(VI) Compounds Mediate Mild Oxidative Bromination

6.1 INTRODUCTION

Bromination of organic substrates, particularly aromatics, has been attracting considerable contemporary interest[1-8] mainly due to the commercial importance of such compounds[9]. Manufacture of a range of chemicals, including antibacterial and antifungal drugs, agrochemicals, flame-retardants and dyes involves bromination[10]. Traditional bromination methods require the use of elemental bromine and solvents, which are environmentally hazardous[11]. Besides elemental bromine, *N*-bromosuccinimide (NBS)[12,13], *N*-bromoacetamide (NBA)[14], or bromodimethylsulfonium bromide[15] could act as stoichiometric brominating reagents but they are expensive and generate organic waste[7]. In this context, a growing ecological awareness among chemists has coincided with an increased understanding of oxidative bromination in biological systems, which has boosted research in the field of oxidative bromination[16].

Vanadium Bromoperoxidases (V-BPO), the enzyme, present in several marine organism, involved in the biosynthesis of a variety of naturally occurring brominated products, catalyze bromination by using H_2O_2 and bromide salts instead of Br_2[11,17]. By itself, H_2O_2, a "green" oxidant, is capable of oxidizing bromide in highly acidic medium (pH<3) but is ineffective in solution at pH > 5.0. The enzyme functions explicitly in catalyzing rate determining bromide oxidation to generate an oxidized bromine species capable of transferring bromine atoms to acceptor molecules with electron rich π bonds[18].

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[17,19] 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[18-24] and to generate environmentally benign catalytic systems for synthesis of brominated organics[8,20]. The credit for first biomimetic functional model of bromoperoxidase goes to Sakurai and Tsuchia[25]. They found bromination of an acceptor occurred in phosphate buffered medium (pH 6.0) containing excess H_2O_2 and KBr in presence of vanadyl sulfate, but not vanadate. In the subsequent years, a large number of vanadium based functional models have been synthesized[21,23,24,26]. However, contrary to natural vanadium bromoperoxidase, 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. Apart from vanadium, oxidation of bromide by hydrogen peroxide has been reported to be catalyzed by tungstate(VI) systems in acidic medium[18-25,27,28]. Jacobs and co-workers reported catalytic systems consisting of tungstate-exchanged layered double hydroxide as heterogeneous catalyst in the oxidative bromination of phenol red and olefines by H_2O_2[7,8,20]. They have also found that W(VI) catalyst are more effective than Mo(VI) and V(V) compounds. Despite the number of reports dealing with the activity of pW compounds as stoichiometric or catalytic oxidants in organic oxidations[29-31], a perusal of literature shows that examples of discreet peroxotungstate compounds displaying activity in oxidative bromination is scanty [32].

It is notable in this context that, a series of dinuclear pV compounds with the distinctive feature of having a μ-peroxo group of the type $[V_2O_2(O_2)_3(L)_3].H_2O$[33-35] (L = asn, gln, gly-gly, gly-ala or gly-asn) as well as pW complexes of the type

$Na_2[W_2O_3(O_2)_4(glycylglycine)_2].3H_2O$[36], $Na_2[W_2O_3(O_2)_4(glycyl\text{-}leucine)_2].3H_2O$[36] and $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$[36], reported previously from our laboratory, 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[33-36].

The above positive findings provided impetus for undertaking the present work. It was envisaged that attachment of pW to polymer support might add advantages of polymeric reagent such as stability and enhanced oxidant activity to the potential ability of the active pW species, leading to development of more efficient reagents or catalysts for bromide oxidation under mild condition. Having gained an access to a number of polymer supported pW compounds, as mentioned in Chapter 3, a particular concern was to assess whether these pW containing macro complexes could act as effective oxidant of bromide in aqueous medium at physiological 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[37,38]. There appears to be no report available on studies dealing with the oxidative bromination of organic substrates by pW complexes anchored to soluble polymeric support.

Chapter **5** of the thesis presents the results of our investigation on reactivity of water soluble polymer bound pW compounds, $[WO(O_2)_2(carboxylate)]$–PA (PA = poly(sodium acrylate) **(PAW) (3.2),** $[WO(O_2)_2(carboxylate)]$–PMA [PMA = poly(sodium methacrylate)] **(PMAW) (3.3),** $[WO(O_2)_2(amide)]$–PAm [PAm = poly(acrylamide)] **(PAmW) (3.4)** and $[WO(O_2)_2(sulfonate)]$–PS [PS = poly(sodium vinyl sulfonate)] **(PSW) (3.5)** in oxidative bromination. We also report here the findings of our study on activity of the insoluble polymer bound pW compound, $[WO_2(O_2)(CN)_2]$—PAN

[PAN = poly(acrylonitrile)] **(PANW) (3.1)** as heterogeneous catalyst in bromination of organic substrates by H_2O_2.

6.2 EXPERIMENTAL SECTION

6.2.1 Measurement of bromination activity in solution

The method of de Boer et al.[39] 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. In case of water soluble polymeric compounds, viz. **PAW (3.2)**, **PMAW (3.3)**, **PAmW (3.4)** or **PSW (3.5)** 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.

In case of insoluble compound, **PANW (3.1)** the compound (5.56 mg, 0.08 mmol W) and H_2O_2 (16 – 80 mM) were added to the reaction mixture containing phosphate buffer (50 mM, pH 5.5), KBr (2 M) and phenol red (1.6 mM). The volume of the reaction mixture was kept at 25 mL. The redox activity was monitored by measuring the change in the absorbance at 592 nm at 30 °C by withdrawing required amount of aliquots from the reaction mixture and diluting it to 100 times at an interval of 5 minutes.

6.2.2 Bromination of organic substrates and product analysis

6.2.2.1 Bromination activity of PAW (3.2), PMAW (3.3), PAmW (3.4) and PSW (3.5)

In a representative procedure, substrate (0.5 mmol) was added to a solution of acetonitrile:water (1:1, 5 mL) containing Et_4NBr (1 mmol) in a 50 mL two necked round-bottomed flask. A weighed amount of solid polymeric compounds, **3.2-3.5** maintaining substrate:pW at 1:1 was then added to the reaction mixture at room temperature under continuous stirring. The progress of the reaction was monitored by TLC. 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 (See **Appendix: 6A**).

6.2.2.2 PANW (3.1) catalysed bromination with H_2O_2

Organic substrate (0.36 mmol) was added to a solution of acetonitrile:water (1:1, 3 mL) containing KBr (0.72 mmol) and 30% H_2O_2 (0.72 mmol) in a 50 mL two necked round-bottomed flasks. A weighed amount of the solid **PANW (3.1)** (0.1 g, 0.036 mmol W) maintaining W:substrate:H_2O_2:Br at 1:10:20:20 was then added to the reaction mixture at room temperature under continuous stirring. After completion of the reaction, the spent catalyst was separated by filtration. The reaction product as well as unreacted organic substrates was isolated, purified and characterized by methods similar to those mentioned above.

6.2.2.3 Regeneration

The regeneration and reusability of the water soluble stoichiometric reagents viz., **PAW (3.2)**, **PMAW (3.3)**, **PAmW (3.4)** and **PSW (3.5)** were tested by using p-nitroaniline as model substrate. In the typical procedure, the reaction mixture contained

the same recipe mentioned above with a 4-fold increase in the amounts of the components. In order to regenerate the compounds, **3.2-3.5** 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 W:peroxide at 1:4 followed by addition of excess DMF with constant stirring until a white pasty mass separated out. After allowing it to stand for 10 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 white microcrystalline solid. The product was separated by centrifugation, washed with cold acetone and dried *in vacuo* over concentrated sulfuric acid.

Regeneration of the insoluble compound, **PANW (3.1)** was achieved by separating the spent catalyst, after completion of the reaction, by centrifugation followed by washing with acetone and drying in vacuo over concentrated sulfuric acid. The catalyst was then placed into a fresh reaction mixture containing salicylaldehyde (0.36 mmol), KBr (0.72 mmol) and 30% H_2O_2 (0.72 mmol) in acetonitrile:water (1:1, 3 mL). The process was repeated up to 6^{th} reaction cycle.

6.3. RESULTS AND DISCUSSION

6.3.1 Bromination in aqueous solution

In recent years significant progress in the design of water soluble catalysts and reagents was achieved, as water appears to be a prospective environmentally friendly solvent for "green" chemistry purposes[40]. Also a number of studies were carried out that used biomimetic approaches for construction of synthetic macromolecular enzyme

models[40-43]. Water, being the native medium for enzymes, is the solvent of choice for such studies[40].

The bromination of phenol red to its tetra brominated product, bromophenol blue was used to measure the bromination activity of the water soluble diperoxotungsten complexes **3.2-3.5** as well as insoluble monoperoxotungsten **PANW (3.1)**, in solution. Phenol red acts as an efficient trap of active bromine species and undergoes stoichiometric bromination reaction, which can be monitored conveniently using electronic spectroscopy (Fig.6.1).

Addition of freshly prepared aqueous solution of each of the compounds **3.2-3.5**, at concentrations indicated (Table 6.1), to the standard reaction of bromide in phosphate buffer in presence of phenol red resulted in gradual color change of the solution from yellow to blue. The spectrum recorded showed a peak at A_{592} characteristic of the product bromophenol blue and a decrease in absorbance of the peak at A_{433} due to loss of phenol red (Fig. 6.1 and Fig. 6.2). The data in Table 6.1 show that soluble macromolecular complexes, **3.2-3.5** possess bromination activity. It was interesting to note that **PANW (3.1)** as such was totally inactive in bromination.

A similar reaction when carried out in absence of phenol red displayed a peak at 262 nm with a shoulder at 237 nm on addition of solutions of compounds (Fig. 6.3). Addition of phenol red to this solution resulted in the decrease in A_{262} nm and a peak at 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[17].

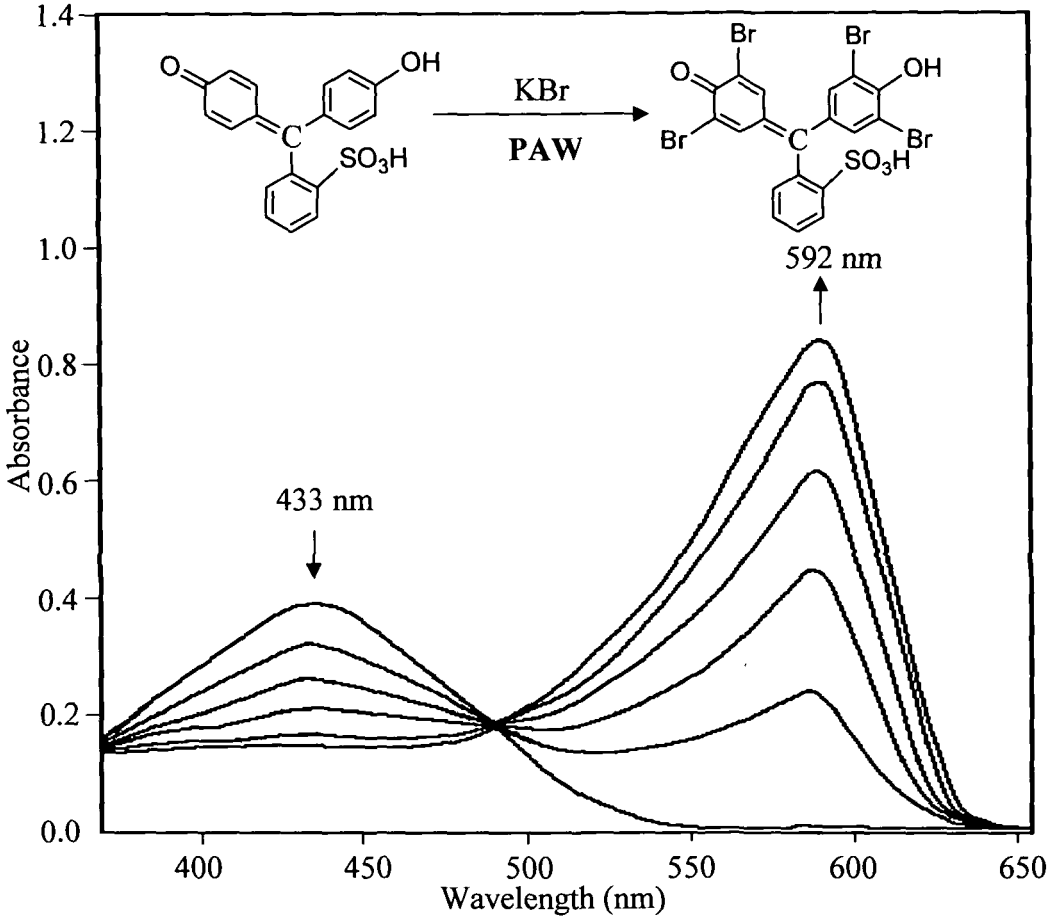


Fig. 6.1 Bromination activity with **PAW (3.2)**. Spectral changes at 2 min interval following bromination of phenol red to bromophenol blue on addition of compound solution to the reaction mixture containing phosphate buffer (0.05 M, pH 5.5), KBr (1 M) and phenol red (20 μM) and **PAW** (0.05 mM of pW).

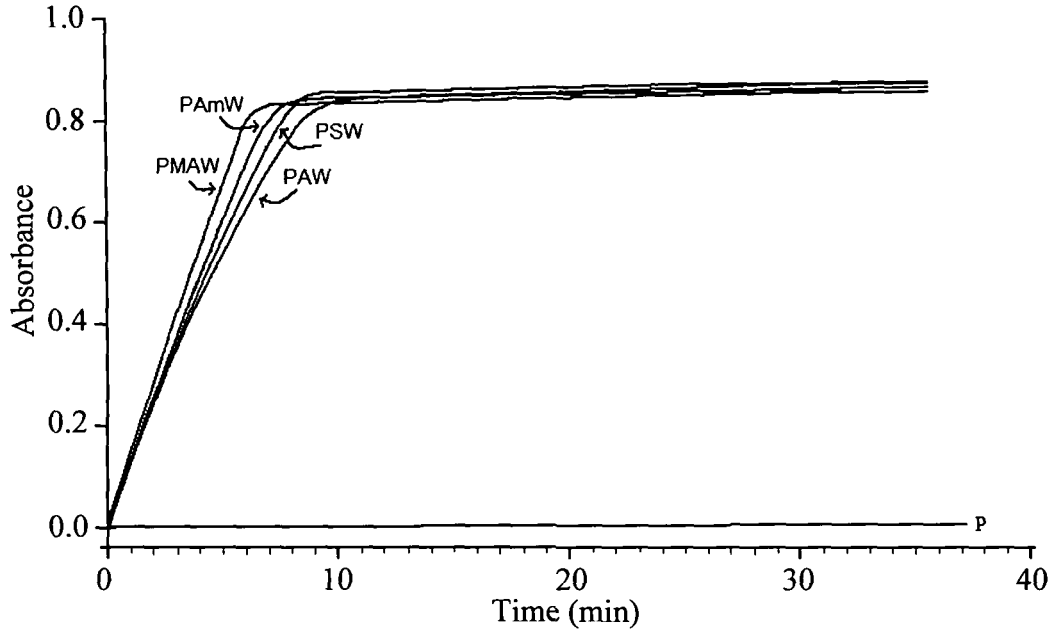


Fig. 6.2. Bromination activity with **PAW (3.2)**, **PMAW (3.3)**, **PAmW (3.4)**, **PSW (3.5)** and free polymer (P). The reaction mixture contained phosphate buffer (0.05 M, pH=5.5), phenol red (20μM), KBr (1 M), and compound (0.05 mM of pW).

Table 6.1 Bromination of phenol red with peroxotungstate complexes **PAW (3.2)**, **PMAW (3.3)**, **PAmW (3.4)** and **PSW (3.5)**.

Compound	Concentration (mM W)	Rate of bromine transfer		Total bromine transfer (Extrapolated to 1mM compound)
		ΔA_{592}/min	μM Br/min	mM Br / mM W
PAW	0.05	0.129	7.65	0.99
PMAW	0.05	0.144	8.54	0.97
PAmW	0.05	0.138	8.19	0.99
PSW	0.05	0.132	7.83	0.99
PANW	0.05	No reaction		

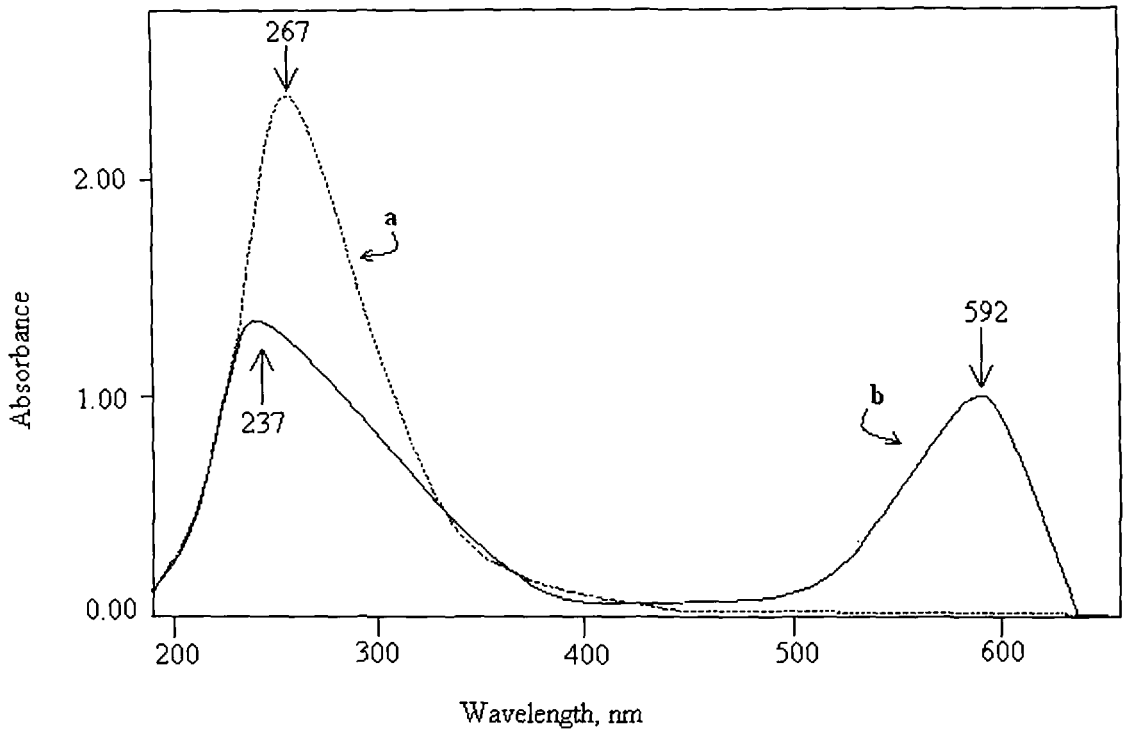


Fig. 6.3 Spectral changes following bromination of phenol red to bromophenol blue on addition of **PAW** (**3.2**). The reaction mixture contained phosphate buffer (0.05 M, pH 5.5), KBr (1 M) and phenol red (20 μM) **PAW** (0.05 mM of pW).(a) KBr + compound in absence of phenol red (b) KBr + compound + phenol red.

It was of significance to note that the bromination activity of each of the polymeric compounds, **3.2-3.5** with anchored diperoxotungsten groups tested, were recorded to be limited to *ca.* 50% of that expected on the basis of the total number of peroxo groups present in the complexes (Table 6.1). The observation is consistent with our earlier findings on oxidant activity of free monomeric or dimeric diperoxotungsten compounds in oxidative bromination[36] and their oxidant activity with respect to organic sulfides (*vide* Chapter 5). Decrease or increase in concentrations of the compounds, substrate or KBr in the reaction solution had no effect on this feature.

6.3.1.1 Effect of pH

The bromination activity of the compounds was surprisingly, found to be maximum at pH 7.0 (Fig 6.4) unlike in the case of peroxovanadate catalyzed oxidative bromination where the rate was reported to increase monotonously with increasing acidity of the reaction medium[44]. We have obtained the data in Table 6.1 at pH 5.5 since the use of this method is limited to pH range near 5.0 in order to achieve conversion from phenol red (pKa 7.9) to bromophenol blue (pKa 4.0). Omission of phosphate buffer from the reaction medium did not alter the bromination activity of the complexes. This indicated that the presence of phosphate was not essential for such activity of the peroxotungsten compounds.

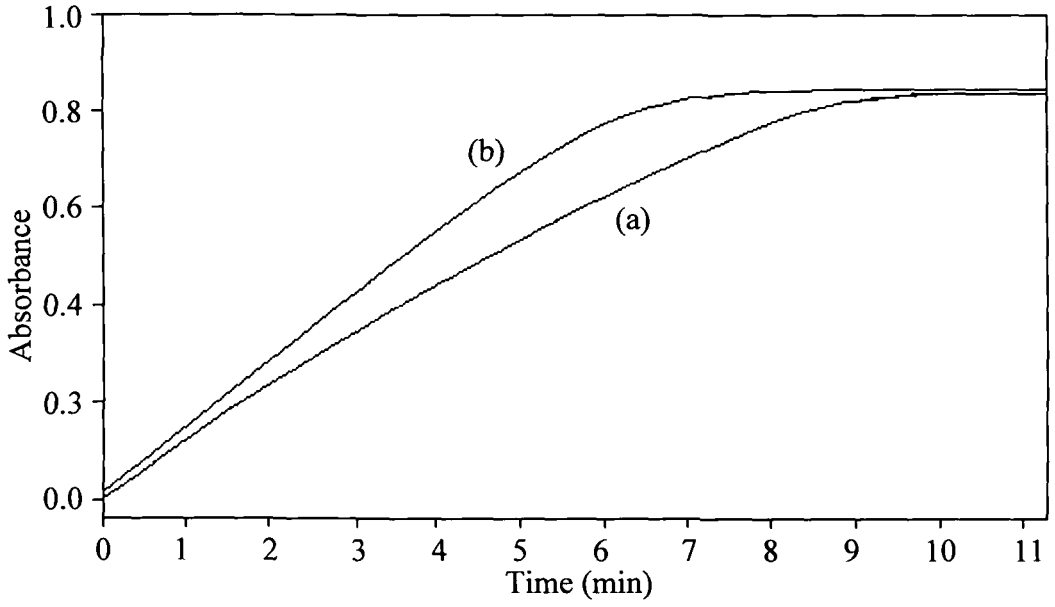


Fig. 6.4 The increase of absorbance at 592 nm indicating the rate of bromination with peroxotungsten compound **PAW (3.2)** in phosphate buffer (0.05 M) of pH 5.5 (a) and pH 7.0 (b). The reaction mixture contained KBr (1 M), phenol red (20 μM) and compound **PAW** (0.05 mM of pW).

6.3.1.2 Effect of H_2O_2 on peroxotungstate mediated bromination

The effect of H_2O_2 on the bromination reaction under standard assay condition was tested. No extra addition of H_2O_2 is required for the stoichiometric bromination reaction of the substrate by the pW compounds, **3.2-3.5** as evident from the data in Table 6.1. While the initial addition of H_2O_2 (0.5 mM) to the reaction solution had no observable effect on the initial rate of bromination (Fig.6.2), it was quite intriguing to note a revival of the bromination activity on addition of H_2O_2 (0.5 mM), after bromination to a spent reaction mixture, which contained excess bromide and substrate. It is reasonable to assume that an inactive tungsten intermediate formed after completion of the stoichiometric bromination process probably combines with peroxide in presence of H_2O_2 to regenerate the respective active brominating species giving rise to a catalytic cycle. Exogenous hydrogen peroxide is therefore required to revive the tungsten complexes to their active forms in order to obtain a catalytic cycle.

6.3.1.3 PANW (3.1) as a catalyst in H_2O_2 mediated bromination in water

It was interesting to note that **PANW (3.1)** which is inactive in bromination on its own, efficiently catalysed the oxidation of phenol red to bromophenol blue in conjunction with H_2O_2 as the terminal oxidant with a TOF of 26.67 h^{-1}. Molar ratio of W:PR was maintained at 1:20 for the catalytic reaction and H_2O_2 was used in 10 fold excess to the substrate PR. The initial rate of bromine transfer for the reaction was recorded to be 1.14 min^{-1} (Fig. 6.5 and Fig. 6.6). The catalyst could be regenerated in situ and recycled after completion of each cycle of bromination by adding a fresh lot of phenol red, bromide and H_2O_2.

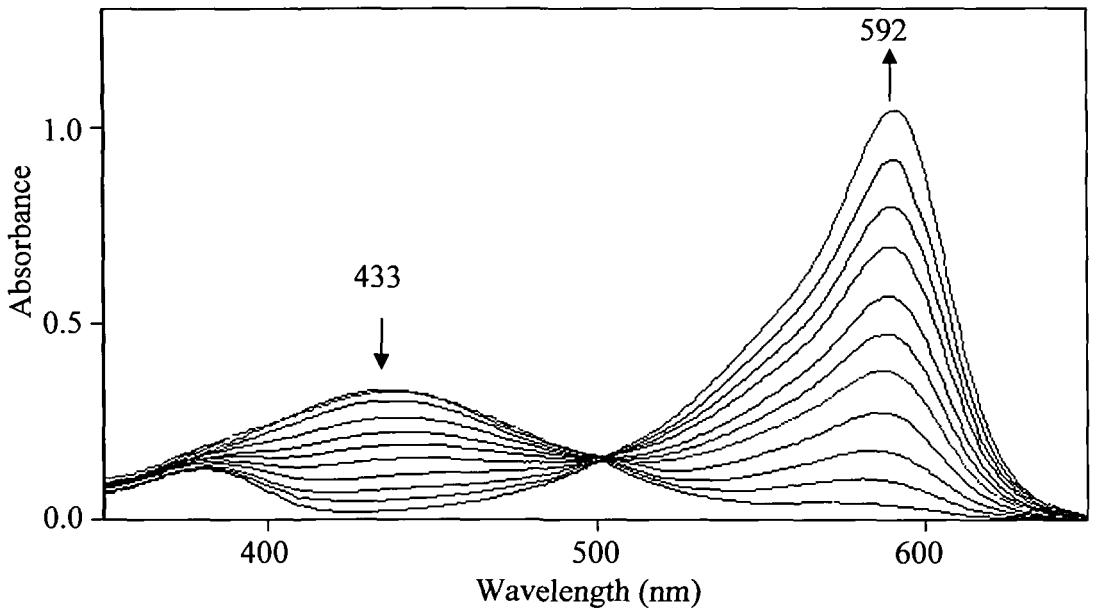


Fig. 6.5 PANW (3.1) catalysed bromination with H_2O_2. The reaction mixture contained phosphate buffer (0.05 M, pH 5.5), KBr (2 M), phenol red (1.6 mM), **PANW** (0.08 mM W) and H_2O_2 (80 mM). Absorbance was recorded by withdrawing required amount of aliquots from the reaction mixture and diluting it to 100 times at an interval of 5 minutes.

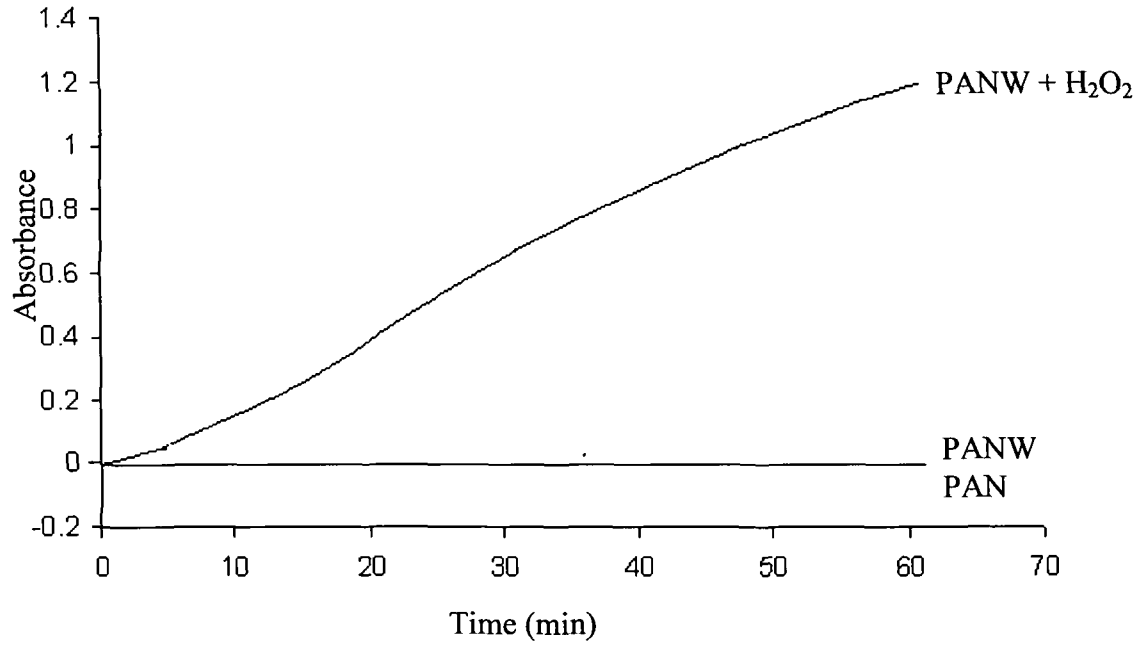


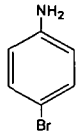
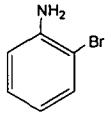
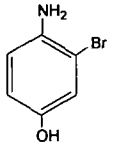
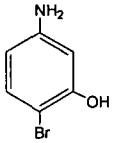
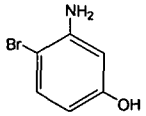
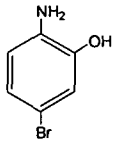
Fig. 6.6 Rate of bromination with **PANW** + H_2O_2. **PANW** and PAN were ineffective in bromination. Absorbances were recorded at 592 nm. The reaction mixture contained phosphate buffer (0.05 M, pH 5.5), KBr (2 M), phenol red (1.6 mM), **PANW** (0.08 mM W) and H_2O_2 (80 mM). Absorbance was recorded by withdrawing required amount of aliquots from the reaction mixture and diluting it to 100 times at an interval of 5 minutes.

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

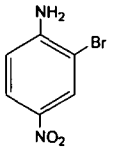
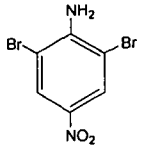
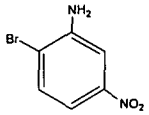
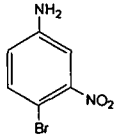
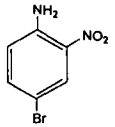
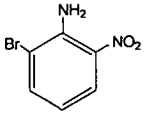
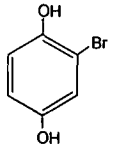
Efficacy of each of pW complexes in mediating bromination of organic substrates in presence of bromide in aqueous-organic media has been explored. Brominations of several activated aromatics into their corresponding bromo-organics were achieved simply by stirring a solution of the substrate in presence of each of the water soluble polymeric reagents **3.2-3.5** in CH_3CN:H_2O (1:1) for 6-7 h at ambient temperature. Results obtained are presented in Table 6.2. Tetraethyl ammonium bromide (Et_4NBr) was used as source of bromide although KBr was also found to be effective as bromide source. 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 or the normal pH of the reaction mixture, and no extra addition of acid or H_2O_2 is required for the stoichiometric bromination reaction of the substrate. Attempted bromination using insoluble complex containing monoperoxotungsten(VI) complex, **PANW (3.1)** under similar reaction condition did not yield brominated products as anticipated.

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. 6.7). Bromination through radical reaction would have produced benzyl bromide instead of bromo-methoxy toluene[23].

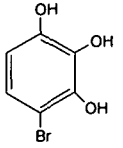
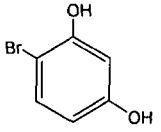
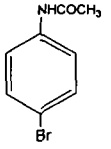
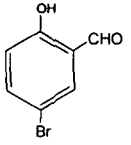
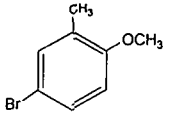
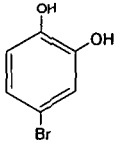
Table 6.2. Bromination of organic substrates mediated by **PAW (3.2)**, **PMAW (3.3)**, **PAmW (3.4)** and **PSW (3.5)** [a]

Entry	Substrate	Product	PAW[a]		PMAW[b]		PAmW[c]		PSW[d]	
			Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)
1	Aniline		6	71	5.5	79	6.5	70	6	76
				11		8		12		10
2	p-aminophenol		7	80	6.5	86	7.5	76	6.5	87
3	m-aminophenol		6	67	6	73	7	81	6	74
				12		11		8		16
4	o-aminophenol		7	83	6.5	78	7.5	85	7	82

Continued...

Entry	Substrate	Product	PAW[a]		PMAW[b]		PAmW[c]		PSW[d]	
			Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)
5	p-nitroaniline		7	68	7.5	74	8	84	7	81
				14		11		6		8
6	m-nitroaniline		6.5	63	7	75	7.5	74	7	71
				17		10		11		13
7	o-nitroaniline		8	65	7.5	73	8	78	7.5	70
				14		12		9		11
8	Quinol		7	86	6.5	81	7.5	87	7	85

Continued...

Entry	Substrate	Product	PAW[a]		PMAW[b]		PAmW[c]		PSW[d]	
			Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)
9	Pyrogallol		6.5	81	6	79	7	86	6.5	84
10	Resorcinol		7	79	7	82	8	85	7.5	86
11	Acetanilide		6	85	6.5	81	7	83	7	89
12	Salicylaldehyde		6	77	6.5	82	7	79	6.5	81
13	o-methoxytoluene		5	90	5.5	81	6	85	5.5	87
14	Catechol		6	84	6.5	87	7	82	7	83

[a]All reactions were carried out with 0.5 mmol substrate, 1.0 mmol TEAB and compound (containing 0.5 mmol W) in CH_3CN/H_2O (1:1, 5 mL) at RT.

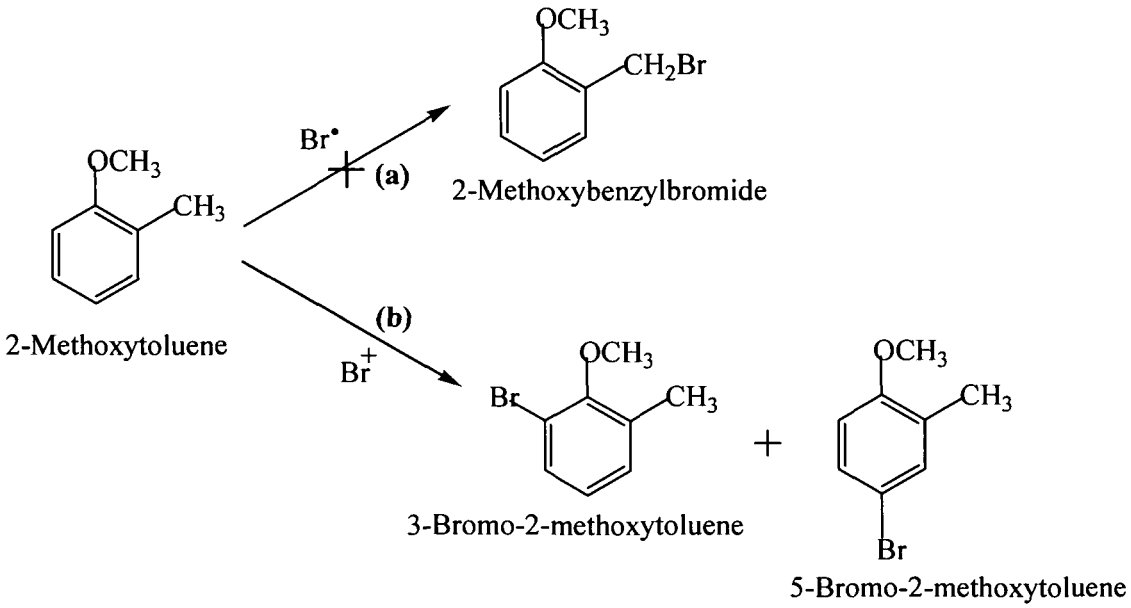


Fig. 6.7 Bromination reaction of 2-methoxytoluene. (a) Possible product of bromination through radical reaction, (b) electrophilic bromination involving 'Br^+' forms exclusively ring substituted products. Bromination reaction using insoluble and water soluble pW compounds produces exclusively bromomethoxytoluene.

6.3.2.1 Catalytic activity of the supported complexes in H_2O_2 induced bromination

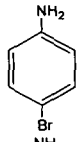
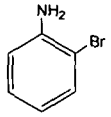
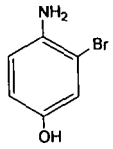
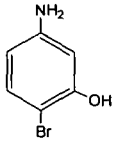
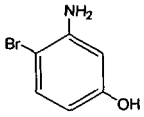
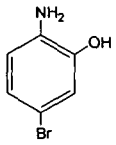
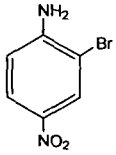
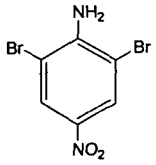
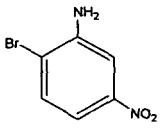
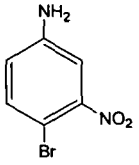
We have further examined the catalytic activity of the supported pW complexes in oxidative bromination of organic substrates by using H_2O_2 as a terminal oxidant. Data presented in Table 6.3 show that **PANW (3.1)** effectively catalyzed the oxidative bromination of organic substrates by H_2O_2 in CH_3CN:H_2O(1:1) to afford the respective bromo-organics with a reasonably good TOF. Molar ratio of W:substrate, substrate:bromide and bromide:H_2O_2 at 1:10, 1:2 and 1:1, respectively was found to be optimum for the desired conversions. On the other hand, the homogeneous analogues displayed poor catalytic activity under identical conditions as has been observed in the reactions conducted in aqueous medium.

6.3.3 Regeneration

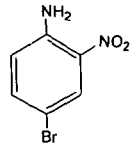
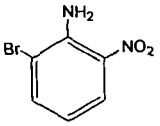
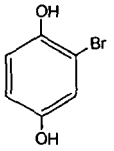
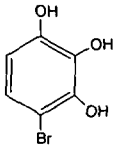
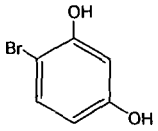
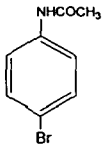
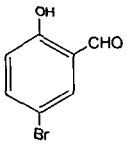
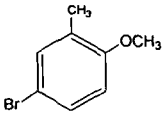
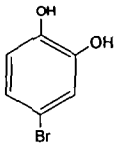
Most frequently, recovery of the soluble polymer supported reagent can be achieved by diluting the water soluble polymer solution by the addition of an excess of a poor solvent which induces precipitation of polymeric species[45,46]. The resulting heterogeneous mixture is filtered to obtain the polymer. For satisfactory recovery of a soluble polymer from reaction mixture proper choice of solvent and temperature are crucial.

The regeneration of water soluble compounds viz. **PAW (3.2)**, **PMAW (3.3)**, **PAmW (3.4)** and **PSW (3.5)** was accomplished by treating the aqueous extract of the spent reaction mixture with H_2O_2, maintaining the peroxide:W 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

Table 6.3 Bromination of organic substrates catalyzed by **PANW (3.1)** with H_2O_2 [a]

Entry	Substrate	Product	Time	Yield (%)	TOF[b] (h^{-1})
1	Aniline		15 min	83	40.0
				12	
2	p-aminophenol		60 min	96	9.6
3	m-aminophenol		40 min	68	14.1
				26	
4	o-aminophenol		60 min	93	9.3
5	p-nitroaniline		90 min	58	6.6
				38	
6	m-nitroaniline		70 min	70	8.5
				25	

Continued...

Entry	Substrate	Product	Time	Yield (%)	TOF[b] (h^{-1})
7	o-nitroaniline		80 min	80	7.5
				16	
8	Quinol		3 h	95	3.3
9	Pyrogallol		60 min	94	10.0
10	Resorcinol		5 h	96	2.0
11	Acetanilide		3 h	96	3.3
12	Salicylaldehyde		4 h 4 h	93 90[c]	2.5 2.5
13	o-methoxytoluene		60 min	91.2	10.0
14	Catechol		3.5 h	81	2.0

[a]Reaction conditions: **PANW** (0.1 g, 0.036 mmol W), Substrate (0.36 mmol), acetonitrile/water (1:1, 3 ml), KBr (0.085 g, 0.72 mmol), 30% H_2O_2 (0.081 mL, 0.72 mmol) at RT.

[b]TOF (Turnover frequency) = mmol of product per mmol of catalyst per hour.

[c]Yield of 6th reaction cycle.

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. 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.

The heterogeneous catalyst, **PANW (3.1)** could be recycled without further conditioning, after separating it from the spent reaction mixture after completion of each cycle of bromination, by adding a fresh lot of substrate, bromide and H_2O_2. It has been found that the catalyst remains effective upto a minimum of six reaction cycles without further treatment with any reagent (Table 6.3, entry 12). However, it is notable that, the activity of the catalyst was observed to decrease gradually in case of highly basic substrates like aniline and nitroanilines. This may be explained on the basis of the fact that nitrile groups of PAN are susceptible to undergo hydrolysis to form amide at pH > 7.5 [47,48]. The resulting structural change in the polymer pendant functional groups of the catalyst, is likely to lead to leaching of the pW moieties causing a fall in its catalytic activity.

6.3.4 Identification of the inactive intermediate

In order to ascertain the nature of the inactive intermediate formed in solution to the product isolated from the aqueous extract of the reaction mixture, after completion of the reaction, was subjected to IR and elemental analysis. IR spectrum of the products isolated as above resembled closely the spectrum of the corresponding original starting

complex in each case, showing the presence of peroxo, oxo and cordinated polymeric ligands. Elemental analysis and EDX results suggested the presence of one peroxo group per W(VI) indicating the formation of a monoperoxotungsten species in each case. We could also isolate an analogous monoperoxotungsten species from a separate experiment involving complex **3.2** (0.5 mmol) and KBr (2.0 mM) under standard assay condition with phenol red omitted from the reaction medium.

6.3.5 Proposed mechanism

The findings from the afore mentioned investigation demonstrate that the activity of the two classes of supported compounds viz., soluble complexes with diperoxotungsten moieties and insoluble compound with monoperoxotungsten groups in oxidative bromination is distinctly different. A reaction pathway is proposed (Fig. 6.8) to account for the stoichiometric oxidant activity of the diperoxotungsten compounds, **3.2-3.5**. A scheme of reactions is proposed to describe the observed catalytic activity of the **PANW** (**3.1**) in bromination by H_2O_2 (Fig. 6.9).

In Fig. 6.8, shown with **PAW** (**3.2**) as a representative, the compound react with bromide to yield oxidized bromine species, proposed to be an equilibrium mixture of BrOH, Br_2 and Br_3^-, with concomitant formation of intermediate (reaction a). Transfer of the bromine atom to the substrate AH from the bromination competent oxidised bromine intermediate takes place (reaction b). In the presence of excess H_2O_2 the inactive intermediate **II** combines with peroxide to regenerate the starting diperoxotungsten complex **I** giving rise to a catalytic cycle (reaction c). To us it appears that bromide would attack an edge-bound peroxo group in preference to a hepta co-ordinated tungsten centre as observed in some other redox processes involving peroxo compounds of W(VI) and Mo(VI)[32,49,50].

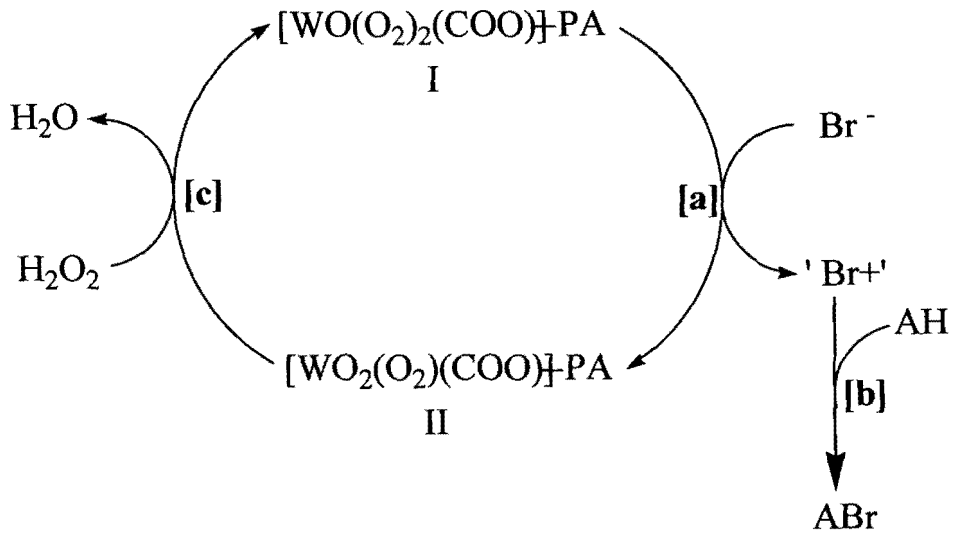


Fig. 6.8 Schematic representation of reactions occurring with water soluble compounds using **PAW (3.2)** as representative. a) Reaction of the diperoxotungstate species **I** with bromide to yield oxidized bromine with concomitant formation of monoperoxotungstate intermediate **II**; b) transfer of bromine from the active species to acceptor AH; c) in presence of excess H_2O_2 the monoperoxotungstate intermediate **II** combines with peroxide to form the original diperoxo species **I**. The monoperoxo species **II** formed combines with H_2O_2 to regenerate the active diperoxotungstate intermediate **I** giving rise to a catalytic cycle.

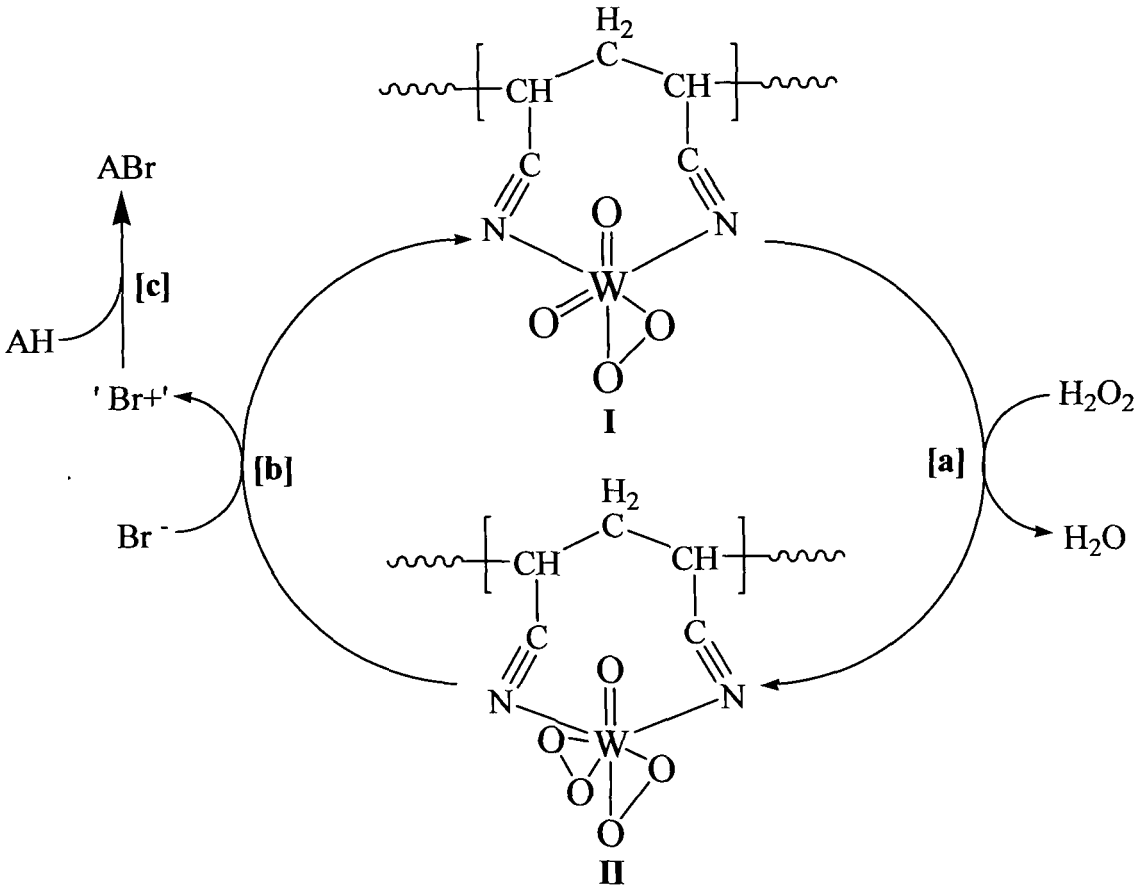


Fig. 6.9 Schematic representation of reactions occurring with **PANW (3.1)**. a) In presence of excess H_2O_2 the monoperoxo species **I** combines with peroxide to form diperoxo tungstate intermediate **II**; b) reaction of the diperoxo intermediate **II** with bromide to yield oxidized bromine; c) transfer of bromine from the active species to acceptor AH. The monoperoxo species **I** formed combines with H_2O_2 to regenerate the active diperoxotungstate intermediate **II** giving rise to a catalytic cycle.

In case of **PANW (3.1)** (Fig. 6.9), first step is likely to be the formation of diperoxotungsten species **II** from species **I**, in presence of H_2O_2 (reaction a). The diperoxo species subsequently oxidises bromine leading to the formation of bromo-organics and regeneration of original monoperoxotungsten catalyst (reaction b and c).

The observed reaction pattern is in accord with the earlier suggestions that for a peroxotungsten complex to be active in oxidation an oxo-diperoxo configuration may be a prerequisite[49]. A monoperoxo Mo(VI) species has been implicated as an intermediate in the mechanisms proposed by Reynolds et al.[32] as well as by Butler and co-workers for the Mo(VI) and W(VI) catalysed bromide oxidations[51]. The results are also consistent with the observation made in case of sulfide oxidation by the compounds **MWG** and **DWG** (*vide* Chapter 5).

6.4 CONCLUSIONS

In summary, the newly synthesised diperoxotungsten complexes supported on soluble polymers could serve as stoichiometric reagent for bromide oxidation at neutral pH. The remarkable feature of the methodology is that no extra addition of acid or H_2O_2 is required for the stoichiometric bromination reaction of the substrate. The immobilized monoperoxotungsten compound **PANW (3.1)**, although was inactive as bromide oxidant under analogous condition, significantly however, in conjunction with H_2O_2 it heterogeneously catalysed oxidative bromination of activated aromatics in aqueous as well as aqueous-organic medium. In view of the environmentally acceptable reaction condition of the bromination protocol mediated by these compounds which also includes redundancy of bromine or hydrobromic acid, the compounds may be considered as

possible candidates of mimic of the function of the enzyme bromoperoxidase. These observations sustain hope that the information generated from the present investigation would find relevance in the context of designing bioinspired synthetic oxidants or catalysts for organic bromination.

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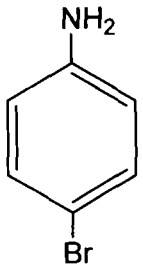
Appendix: 6A Characterization of Brominated products



(a) 4-Bromoaniline :

Isolated as light yellow solid.
mp: 63 °C
^{1}H NMR (400MHz; $CDCl_3$): 7.16(d, 2H, J=7Hz), 6.5(d, 2H, J=7Hz), 3.5 (s, 2H, $-NH_2$)

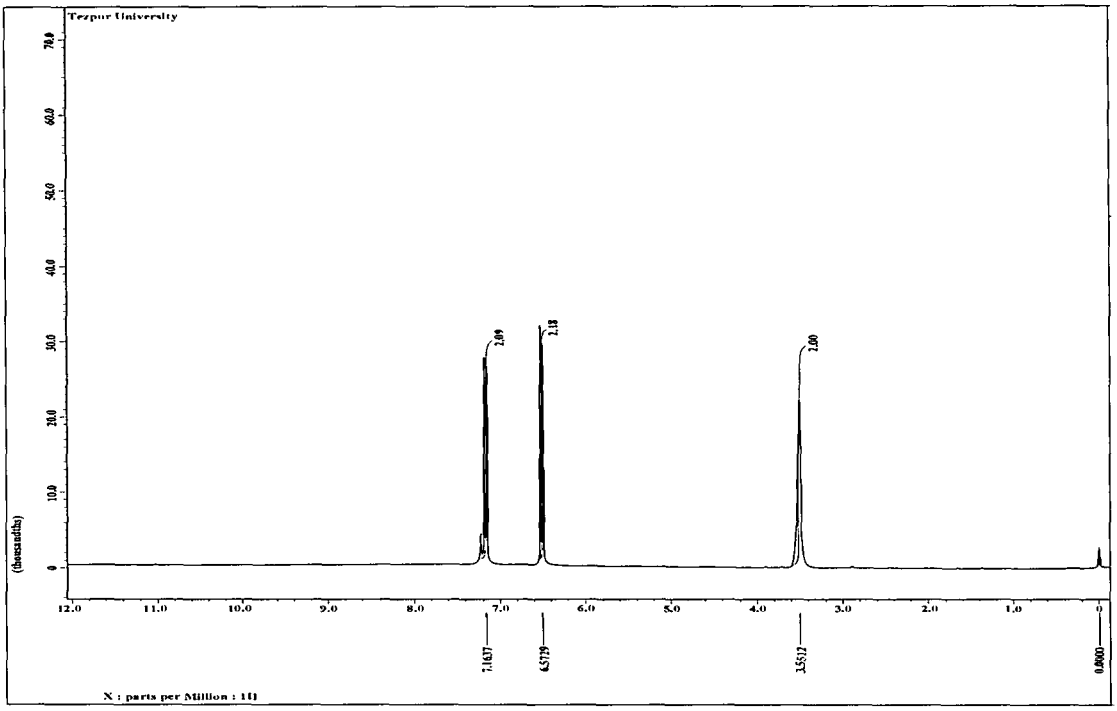
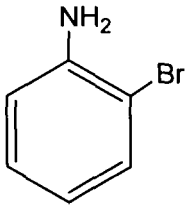


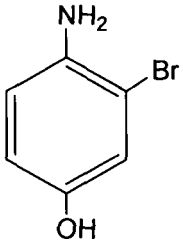
Fig. 6A. 1 ^{1}H NMR spectra of 4-bromoaniline.

(b) 2-Bromoaniline :



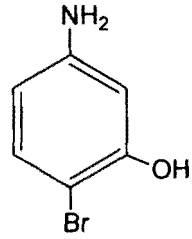
Isolated as light yellow solid.
mp: 31 °C
^{1}H NMR (400MHz; $CDCl_3$): 7.5-6.36 (m, 4H), 4.0 (s, 2H, $-NH_2$)

(c) 4-Amino-3-bromophenol :



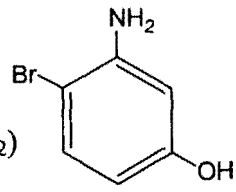
Isolated as yellow solid.
mp: 151 °C
^{1}H NMR (400MHz; $CDCl_3$): 8.3(s,1H), 7.4(d, 1H, J=6Hz), 6.9 (d, 1H, J=6Hz), 6.2-5.9(s, 2H, $-NH_2$)

(d) 5-Amino-2-bromophenol



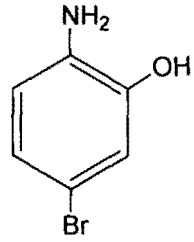
Isolated as light yellow solid.
mp: 139 °C
^{1}H NMR (400MHz; $CDCl_3$): 7.8-7.25(m, 3H), 5.3-5.0(s, 2H, -NH_2)

(e) 3-Amino-4 -bromophenol



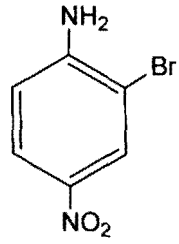
Isolated as yellow solid.
mp: 168 °C
^{1}H NMR (400MHz; $CDCl_3$): 8.1-7.5(m,3H), 5.6-5.3(br, 2H, -NH_2)

(f) 2-Amino-5- bromophenol :



Isolated as light yellow solid.
mp: 137 °C
^{1}H NMR (400MHz; $CDCl_3$): 8.3(s,1H), 7.8(d, 1H, J=6Hz), 7.1 (d, 1H, J=6Hz), 6.5-6.2(s, 2H, -NH_2)

(g) 2-Bromo-4-nitroaniline



Isolated as brown powder.
mp: 104°C
^{1}H NMR (400MHz; $CDCl_3$): 8.25(s,1H), 7.9(d,1H, J=6Hz), 6.64 (d,1H, J=6Hz), 4.8-4.6 (br, 2H, -NH_2)

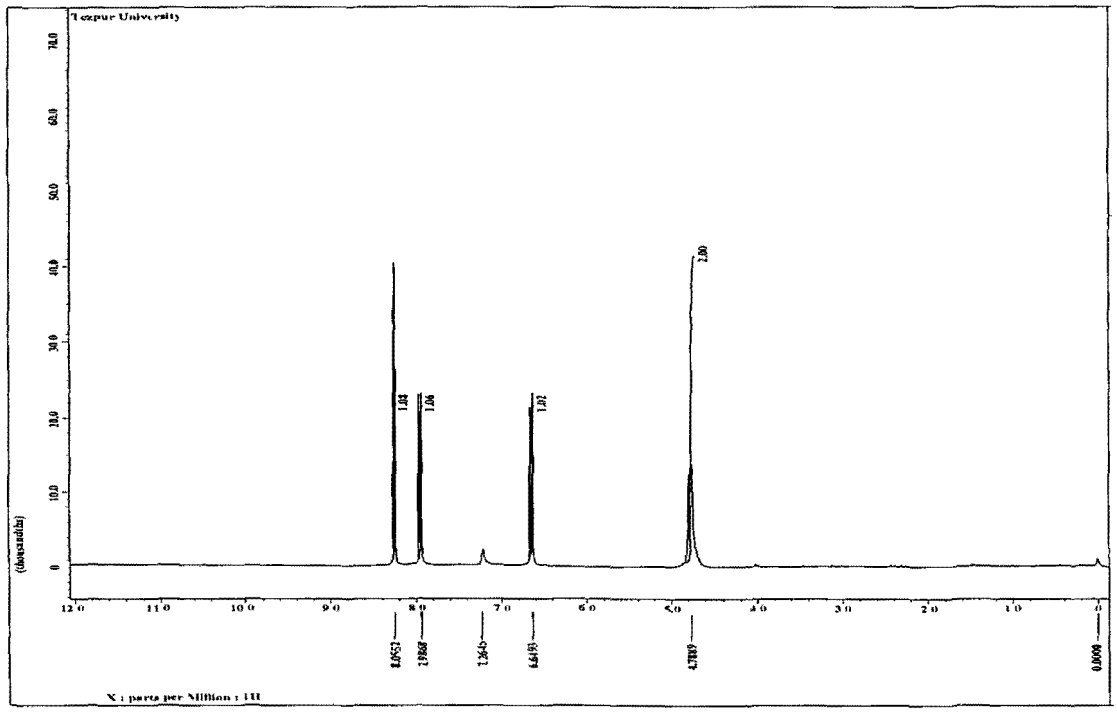
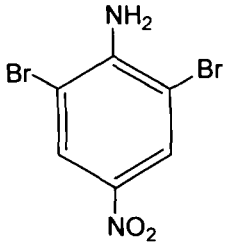


Fig. 6A. 2 ^{1}H NMR spectra of 2-bromo-4-nitroaniline.

(h) 2,6-Bibromo-4-nitroaniline



Isolated as yellow powder.
mp: 208 °C
^{1}H NMR (400MHz; $CDCl_3$): 8.05(s, 2H), 4.9-4.6 (br, 2H, -NH_2)

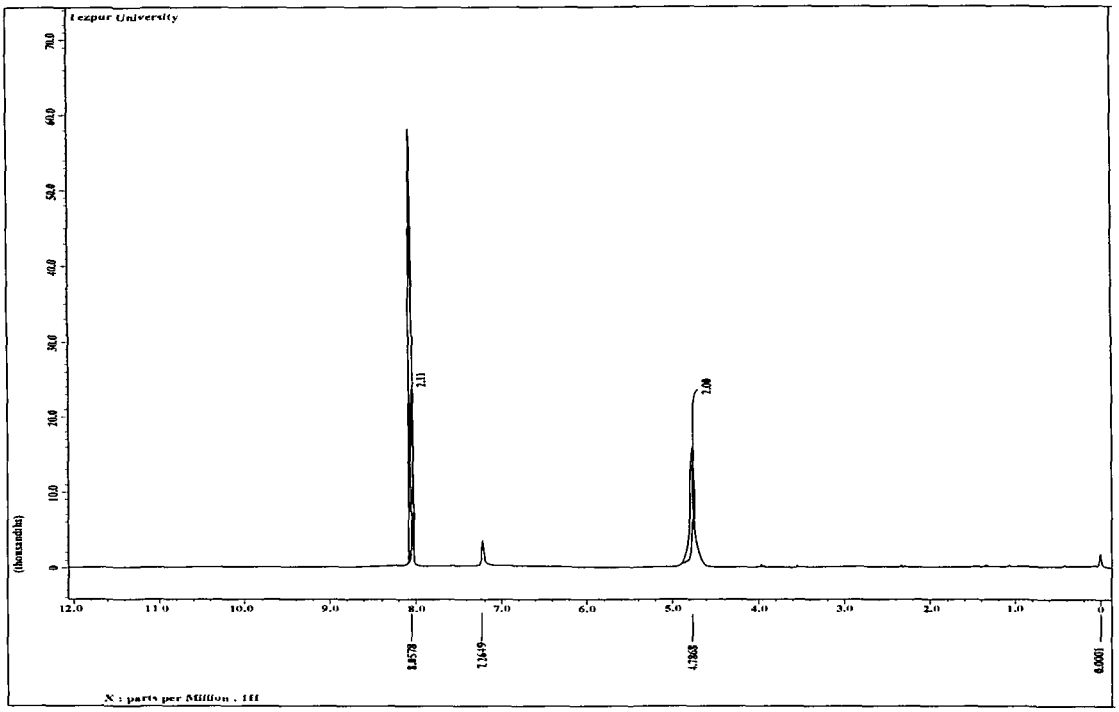
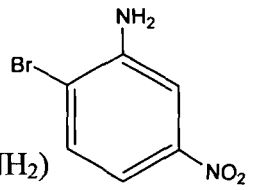


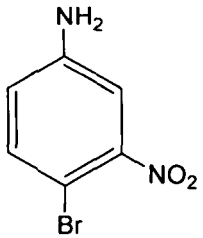
Fig. 6A. 3 ^{1}H NMR spectra of 2,6-Dibromo-4-nitroaniline.

(i) 2-Bromo-5-nitrobenzenamine



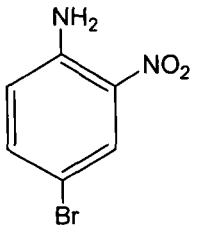
Isolated as dark yellow fine crystalline needles.
mp: 140 °C
^{1}H NMR (400MHz; $CDCl_3$): 7.7-7.25(m, 3H), 4.6-5.0(br, 2H, -NH_2)

(j) 4-Bromo-3-nitroaniline



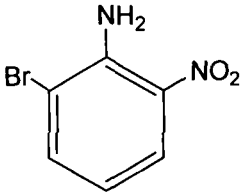
Isolated as orange crystalline powder.
129 °C
^{1}H NMR (400MHz; $CDCl_3$): 7.5-6.7(m, 3H), 4.5-4.8(br, 2H, -NH_2)

(k) 4-Bromo-2-nitroaniline



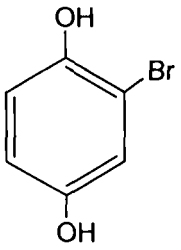
Isolated as solid orange powder.
mp: 110 °C
^{1}H NMR (400MHz; $CDCl_3$): 8.3(s, 1H), 7.45(d, 1H, J=7Hz), 6.75(d,1H, J=7Hz), 6.35-6.05(br, 2H, -NH_2)

(l) 2-Bromo-6-nitroaniline



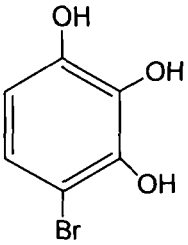
Isolated as orange solid.
mp: 72-74 °C
^{1}H NMR (400MHz; $CDCl_3$): 7.3-7.05(m, 3H), 6.5(s,2H, -NH_2)

(m) 2-Bromobenzene-1,4-diol



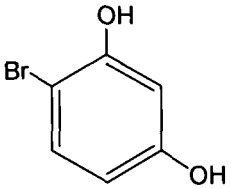
Isolated as brown fine crystalline powder.
mp: 114 °C
^{1}H NMR (400MHz; $CDCl_3$): 8.5(s, 1H), 8.2-7.5(m, 2H)

(n) 4-Bromobenzene-1,2,3-triol



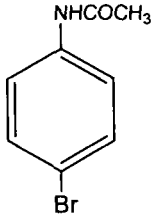
Isolated as brown solid.
mp: 280 °C
^{1}H NMR (400MHz; $CDCl_3$): 6.7 (d, 1H, J=6Hz), 6.4(d, 1H, J=6Hz), 5.3-5.0 (br, 3H, -OH)

(o) 4-Bromobenzene-1,3-diol



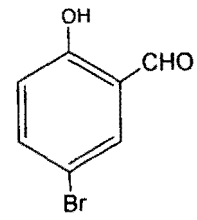
Isolated as pink crystalline powder.
mp: 100 °C
^{1}H NMR (400MHz; $CDCl_3$): 6.9(d, 1H, J=7Hz), 6.3(d, 1H, J=7Hz), 6.1(s, 1H), 5.6-5.2(br, 2H, -OH)

(p) N-(4-bromophenyl)acetamide



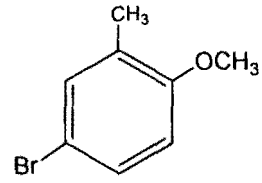
Isolated as off-white powder.
mp: 166 °C.
^{1}H NMR (400MHz; $CDCl_3$): 7.7-7.3(m, 5H), 1.9(s, 3H)

(q) 5-Bromo-2-hydroxybenzaldehyde



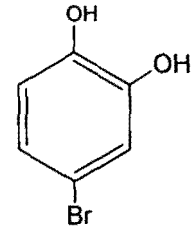
Isolated as slightly yellow powder.
mp: 105 °C
^{1}H NMR (400MHz; $CDCl_3$): 10.0(s, -CHO), 7.9(s, 1H), 7.5(d, 1H, J=6Hz), 6.9(d, 1H, J=6Hz), 5.0(s, 1H, -OH)

(r) 1-Methoxy-2-methyl-4-bromobenzene



Isolated as light yellow solid.
mp: 69 °C
^{1}H NMR (400MHz; $CDCl_3$): 7.9(s, 1H), 7.4(d, 1H, J=6Hz), 6.85(d, 1H, J=6Hz), 3.6(s, 3H, $-OCH_3$), 1.2 (s, 3H, $-CH_3$)

(s) 4-Bromo-1,2-dihydroxybenzene



Isolated as white crystals.
mp: 87 °C
^{1}H NMR (400MHz; $CDCl_3$): 6.9(d, 1H, J=7Hz), 6.7(s, 1H), 6.3(d, 1H, J=7Hz), 5.3-4.9(br, 2H, -OH)

CHAPTER 7

Interaction of Water Soluble Macromolecular Peroxotungsten(VI) Complexes with Enzymes : Catalase, Acid, and Alkaline Phosphatases

7.1 INTRODUCTION

Recent findings on biochemical activity of the compounds of tungsten such as their antiviral[1-3], antiobesity[4], insulinomimetic activity[5-11] and their affinity to inhibit a number of enzyme functions[12,13] including hydrolysis of phosphoproteins[5,14-20], came as exciting contributions to the current knowledge of biological importance of the metal and its compounds. Normoglycemic properties of tungstates have been extensively investigated[15]. It has been demonstrated by Claret et al. that antiobesity effects of tungstate are expressed without any adverse effects such as gastrointestinal discomfort, the main undesirable side effect of vanadate[4,15]. These observations marked this group as promising new therapeutic agents[15].

It has been observed by Shechter et al. that while tungstate and molybdate facilitate bioeffects in rat adipocytes only at higher concentration, pW and pMo present in a solution of the respective metal and H_2O_2 were found to be 80-180 fold more potent stimulator than the corresponding metalloxides[9]. Although the precise mechanisms involved in most of the metabolic actions of the oxo or peroxo derivatives of these metals is still unclear[9,21-23], a definite correlation has been established between abilities of oxy anions of V, Mo and W as well as their peroxo derivatives to inhibit protein phosphatases and their *in vivo* insulin mimetic activities[21-23]. The inhibition of phosphatases by vanadate and pV compounds has been the topic of several reviews[21,24-38]. Moreover, it is

notable that potential usage of compounds of vanadium as therapeutic antidiabetic agents has been extensively investigated in recent years[16,17,39,40], yet only three compounds have been introduced in clinical tests with humans[25,41,42]. This is mainly owing to the fact that most of the synthetic pV compounds suffer from the disadvantages of being hydrolytically unstable or toxic[20,23,40,43-45], limiting their utility as pharmacological agents. It is therefore intriguing that, despite the knowledge that peroxotungstates display superior bio-relevant properties such as higher hydrolytic stability[9], bioavailability and low toxicity compared to pV[15], potential of discreet pW compounds as biologically active agent remains relatively unexplored. As a case in point, no report on direct *in vitro* effect of discreet pW compounds on different enzymes including phosphohydrolases appeared to exist until we reported our observations on the phosphatase inhibitory activity exhibited by heteroligand dinuclear and mononuclear pW compounds, synthesized in our laboratory[46-48]. Inhibitor potency of these compounds were found to be significantly higher than that of tungstate or bare peroxotungstate species formed in solution and appeared to be sensitive to the ligand environment employed[47,48]. Furthermore, to the best of our knowledge, there is still no previous study that reported the effect of pW compounds on acid phosphatases.

The importance of enzyme inhibition as a mode of action for inorganic drugs is being increasingly recognized in recent years[1,15]. In order to gain a better understanding of the mechanism of action of precursor complexes, biological models are being used to clarify potential toxic metal inhibitory effects in main cellular processes[14] . Both mammalian alkaline and acid phosphatases have been implicated in the control of protein phosphotyrosine content, but the specificity of cellular protein phosphatases is poorly understood[49-52].

With the above considerations in mind, and our specific interest on biological activity of peroxotungsten compounds, we deemed it worthwhile to study the effect of the newly synthesised macro complexes on phosphatases vis-a -vis free monomeric as well as dimeric pW compounds. One of the specific interests was to explore whether binding of low molecular weight pW species to macromolecular ligands would alter their affinity as enzyme inhibitor. Two types of membrane associated enzymes viz., wheat thylakoid membrane ACP and rabbit intestine ALP were chosen for our investigation because in addition to being excellent models to investigate toxic metal inhibitory effect in membrane proteins, these enzymes play key roles in a variety of biological phenomena[53-55]. Thylakoid membrane phosphatase is unique to photosynthesis[55-59]. Non-specific alkaline phosphatases (ALP), one of the ubiquitous groups of phosphohydrolases, are used extensively in immunoassays[26,60-64] and are involved in development of several diseases.

Apart from investigating the effect of the macro complexes on activity of phosphatases, we considered it important to examine the interaction of the newly synthesized compounds with the enzyme catalase. Catalase is a powerful reactive oxygen (ROS) mopping enzyme responsible for breakdown of H_2O_2 to H_2O and O_2. H_2O_2 is a significant cellular oxidant needed particularly for action of peroxidases that yield highly active intermediates[65]. In the last two decades, H_2O_2 is being increasingly recognized as a key signal transducing agent regulating a variety of cellular processes[65-68]. Fast decomposition of extracellular H_2O_2 is a constraint for studying the signaling activities of H_2O_2 because cells are equipped with catalase and peroxidase that rapidly deplete H_2O_2[65-67]. In order to investigate how the small concentrations of H_2O_2 generated in cells will function in presence of abundant catalase, it is desirable to have peroxide derivatives easily formed and stable to degradation, yet efficient in their action, that can substitute for

H_2O_2. It is known that the peroxo group in DPV, compared to H_2O_2 is less accessible to degradation by catalase and is active as substrate in horseradish peroxidase reaction in presence of catalase at 1/100 concentration[69]. Implicit in these findings is that complexing with vanadium increases stability of peroxide, at the same time they appear to become more efficient in the form of DPV. Previous studies demonstrated that diperoxovanadate compound can be used as tools in the study of the signaling actions of H_2O_2[65,70]. It is of significance that, some of the heteroligand mononuclear and dinuclear pV and pW complexes synthesized and reported earlier by us showed reasonable resistance to catalase action[46-48,71]. Since the primary objective of our present work has been to generate information on some of the biochemically important features of the complexes it was considered important to examine the fate of the compounds in presence of catalase vis-a-vis H_2O_2, its natural substrate.

Presented in this chapter are the findings of our investigation on the kinetics of inhibition of rabbit intestine alkaline phosphatase (ALP) and wheat thylakoid membrane acid phosphatase (ACP) by the water soluble polymer anchored peroxotungsten compounds viz., $[WO(O_2)_2(carboxylate)]$-PA **(3.2),** $[WO(O_2)_2(carboxylate)]$-PMA **(3.3),** $[WO(O_2)_2(amide)]$-PAm **(3.4)** and $[WO(O_2)_2(sulfonate)]$–PS **(3.5)**. Effect of previously reported monomeric heteroligand peroxotungsten(VI) complex, $[WO(O_2)_2(glycyl\text{-}glycine)(H_2O)].3H_2O$ **(5.1)** and a dimeric compound $Na_2[W_2O_3(O_2)_4(cystine)].4H_2O$ **(5.3)** on ACP activity has been evaluated. Internal comparison were drawn on the effect induced by the two class of compounds viz., supported and free peroxotungstates on the phosphatases. Results of studies on the interaction of the polymeric complexes with the enzyme catalase are also included in this chapter.

7.2 EXPERIMENTAL SECTION

7.2.1 Measurement of acid phosphatase activity

The activity of acid phosphatase was monitored by assay of the p-nitrophenol (p-NP) released from p-nitrophenyl phosphate (p-NPP) at the pH optimum, 4.6 at 30 °C by measuring absorbance at 405 nm. In the standard assay, the reaction mixture contained acetate buffer (0.1 M, pH=4.6), acid phosphatase (18.38 μg protein/mL) and compound solution (concentration varies between 0.1-80 μM) was first pre-incubated at 30 °C for 5 min. Subsequently, the substrate, p-PNPP (2 mM) was added and the mixture was incubated for 30 min at 30 °C and finally the reaction was stopped by adding NaOH solution (0.5 M). The molar extinction coefficient of *p*-nitrophenolate ion, at 405 nm, in alkaline medium is 18000 M^{-1} cm^{-1}. The activity in the absence of inhibitor was considered as control (100%). For the polymer bound compounds concentrations were on the basis of actual peroxometal loading (mmol g^{-1}). All the assays were performed in triplicate. The data in figures are presented as the means ± SE from three separate experiments.

7.2.2 Measurement of alkaline phosphatase activity

Phosphatase activity was assayed spectrophotometrically by using p-NPP as a substrate. The continuous production of p-nitrophenol (p-NP) was determined at 30 °C by measuring absorbance at 405 nm in a reaction mixture containing ALP from rabbit intestine (3.3 μg protein/mL), p-NPP (2 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 pW and bare ligands were assessed by adding different concentrations of each species in the ALP assay. For the polymer bound compounds concentrations were on the basis of actual peroxometal loading (mmol g^{-1}). 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.

7.2.3 Determination of kinetic parameters

The kinetic parameters V_{max} and K_m of an enzyme catalyzed 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 $1/V_0$ (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 $1/V_0$ (Y axis) vs $1/S$ (X axis), X intercept= $1/K_m$, 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.

7.2.4 Effect of catalase on the complexes

The effect of catalase on complexes **3.2-3.5** 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, 90 and 120 min of starting the reaction. The fate of the compounds towards degradation by catalase is also studied by monitoring their electronic spectral band in the range of *ca.* 230-250 nm, which is characteristic of absorbance caused by diperoxotungstate species, at ambient temperature for possible changes.

7.3 RESULTS AND DISCUSSION

7.3.1 Inhibition of acid and alkaline phosphatases by the peroxotungsten compounds

Acid phosphatases from human prostate, wheat germ, and other sources have been isolated as phosphohistidyl enzymes containing an active site histidine[17]. The mammalian enzyme acid phosphatase possess dinuclear iron active sites[53,54] and highly conserved amino acid sequences[72-75]. It catalyzes the hydrolysis of phosphate ester bond at an acidic medium (optimum pH of 4.9-6.0)[54]. The enzyme is found in different forms in different organs, and their serum levels are used to evaluate the success of the surgical treatment of prostate cancer. In the past, they were also used to diagnose this type of cancer[76]. Alkaline phosphatase is a zinc metalloenzyme with a broad substrate specificity, which catalyzes the hydrolysis of organic phosphate monoesters possibly via an enzyme-phosphate intermediate[60,77]. The maximum activity of the enzyme is observed at pH $\geq$ 8[60,61]. Phosphotransferase activity and protein phosphatase activity are some of the other probable functions assigned to the enzyme.

Using the established enzyme assay system and p-NPP as substrate, the effect of the polymer anchored compounds upon the activities of the membrane associated proteins, viz., wheat thylakoid membrane ACP as well as rabbit intestine ALP was

examined vis-a-vis the effect induced separately by the previously reported free mononuclear or dinuclear pW compounds, **MWG (5.1)** and **DWC (5.3)** as well as tungstate anion. The data presented in Fig. 7.1 and 7.2 indicate that the pW compounds irrespective of being polymer bound or free was able to markedly inhibit the activity of each of the model enzymes in a dose dependent manner. The inhibitory potential of the inhibitor species were quantified by determining the half-maximal inhibitory concentration (IC_{50}) for each inhibitor, which gave rise to a 50% suppression of the original enzyme activity (Table 7.1 and 7.2). The IC_{50} values for the polymeric compounds are in terms of their actual peroxometal loading. It is apparent from the data that enzymes have different sensitivity to the inhibitor complexes. It is notable that the ACP activity was efficiently inhibited by the pW compounds at doses lower than 1 μM.

In order to evaluate the mode of inhibitory action of the complexes on the activity of the model enzymes used, the kinetic parameters, K_m and V_{max} were determined in absence as well as in presence of peroxo metal compounds using Lineweaver-Burk double reciprocal plots. Enzyme kinetics investigation is a major tool in enabling us to distinguish between the inhibition mechanisms of enzyme catalyzed reactions. Kinetic measurements for acid phosphatase (ACP) catalysed hydrolysis of p-NPP at several different substrate concentrations in presence of each of the inhibitors yielded straight lines with a point of intersection in the second quadrant (Fig. 7.3 – Fig. 7.9). As demonstrated by the L.B. plots (Fig. 7.3 – Fig. 7.9) an increase in concentration of each of the polymeric complexes led to a substantial decrease in V_{max} although K_m remained unaffected suggesting a non-competitive type of inhibition by these complexes. On the other hand, the free monomeric complex **MWG (5.1)** as well as dinuclear complex **DWG (5.3)** exerted mixed inhibitory effects combining competitive and non-competitive

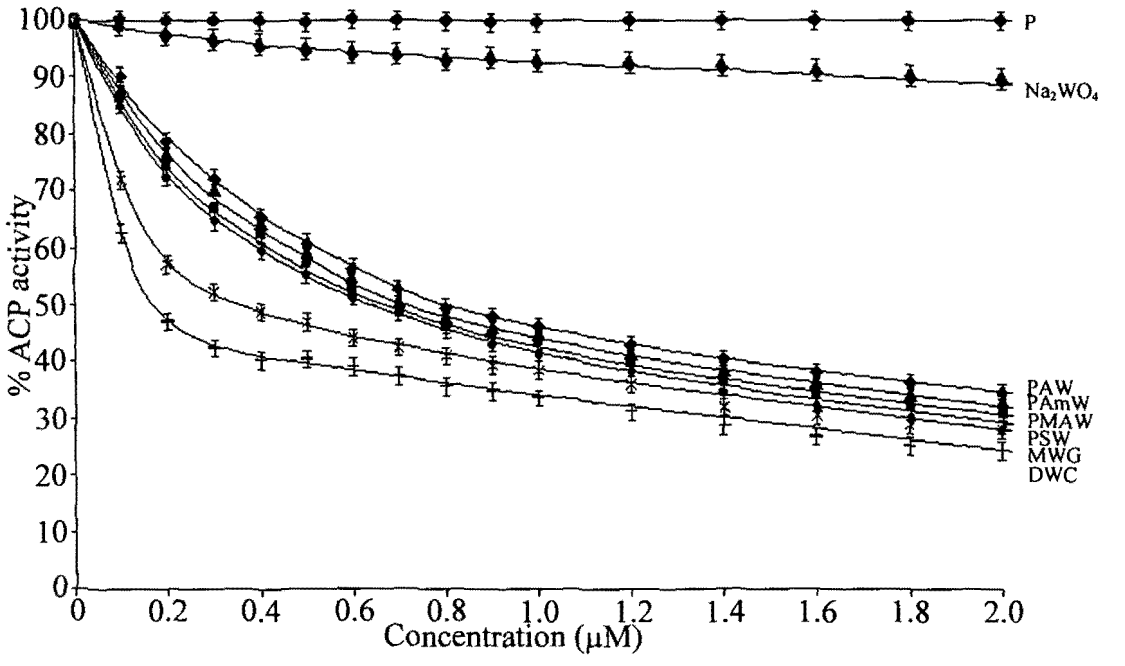


Fig. 7.1 Effect of Compounds **PAW, PMAW, PAmW, PSW, MWG, DWC, Na_2WO_4** and free polymers (**P**) on the activity of ACP. The ACP catalyzed rates of hydrolysis of p-NPP at pH 4.6 were determined at 30 °C by measuring A_{405} in a reaction mixture containing ACP (18.38 μg/mL), p-NPP (2 mM) in acetate buffer (0.1 M, pH=4.6) 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. For polymeric compounds concentrations are on the basis of peroxometal loading.

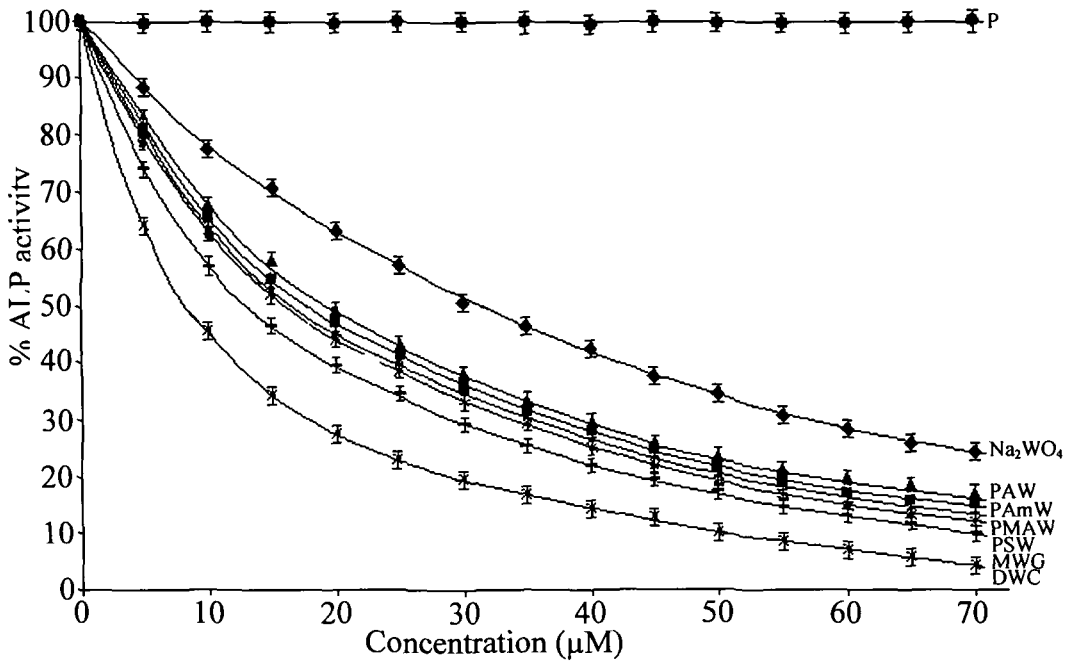


Fig. 7.2 Effect of compounds **PAW**, **PMAW**, **PAmW**, **PSW**, **MWG**, **DWC**, **Na_2WO_4** and free polymers (**P**) on activity of ALP from rabbit intestine. The ALP catalyzed rates of hydrolysis of p-NPP at pH 10.0 were determined at 30 °C by measuring A_{405} in a reaction mixture containing 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. The data are presented as the means ± SE from three separate experiments. For polymeric compounds concentrations are on the basis of peroxometal loading.

modes. Tungstate also exhibited non-competitive type inhibition on ACP activity under these conditions although it was observed to be approximately 20-90 fold less potent compared to the peroxotungstates examined.

It was important to assess the affinity of the enzyme for the inhibitor by determining the inhibitor constants. All compounds exhibited affinity for the acid phosphatase in a close order of magnitude as observed from the K_I and K_{II} values (Table 7.1). 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 $-K_I$ (Fig. 7.3 - Fig. 7.9). 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 $-K_{II}$ (Fig. 7.3 – Fig. 7.9). For each of the polymer-anchored pW complexes, value of K_I was found to be equal to K_{II}, which is typical of a non-competitive inhibitor. For free peroxotungstates, **DWC (5.3)** or **MWG (5.1)**, $K_{II} > K_I$ as is the case with a mixed type of inhibitor with major mode of inhibition being of the competitive type. The inhibitors could thus be arranged in the following order of potency: **DWC > MWG > PSW > PMAW > PAmW > PAW > Na_2WO_4**. Importantly, although the effect of the individual ligand on the enzyme activity is practically negligible under the assay conditions used, our results show that there is a marked influence of the coligand environment on the inhibitor potency of the intact metal complexes. The free heteroligand pW compounds are more effective inhibitors compared to the peroxotungstates in macroligand environment.

The mode of inhibitory action of the polymeric pW compounds on rabbit intestinal alkaline phosphatase was similar to that found for the wheat thylakoid membrane acid phosphatase. However, significant differences were noted in the values of

Table 7.1 Half-Maximal Inhibitory Concentration (IC_{50}) and Inhibitor Constants (K_i and K_{ii}) Values for pW Compounds and Other Inhibitors Against ACP

Sl. No.	Compound	$IC_{50}(\mu M)$	$K_i(\mu M)$	$K_{ii}(\mu M)$	K_{ii}/K_i	Types of inhibition
1	**PAW**	0.8081	0.83	0.82	0.98	Non-competitive
2	**PMAW**	0.665	0.715	0.70	0.97	Non-competitive
3	**PAmW**	0.713	0.775	0.770	0.99	Non-competitive
4	**PSW**	0.641	0.665	0.614	0.92	Non-competitive
5	**MWG**	0.36	0.20	0.56	2.80	Mixed inhibition
6	**DWC**	0.17	0.10	0.34	3.40	Mixed inhibition
7	**Na_2WO_4**	15.23	10.45	10.10	0.96	Non-competitive
8	Free polymer	---	---	---	---	---

Note: The ACP catalyzed rates of hydrolysis of p-NPP at pH 4.6 were determined at 30 °C by measuring A_{405} in a reaction mixture containing ACP (18.38 μg/mL), p-NPP (2 mM) in acetate buffer (0.1 M, pH=4.6) in the presence of stated concentrations of the inhibitors (Fig. 7.3 – Fig. 7.9). For polymeric compounds concentrations are on the basis of peroxometal loading.

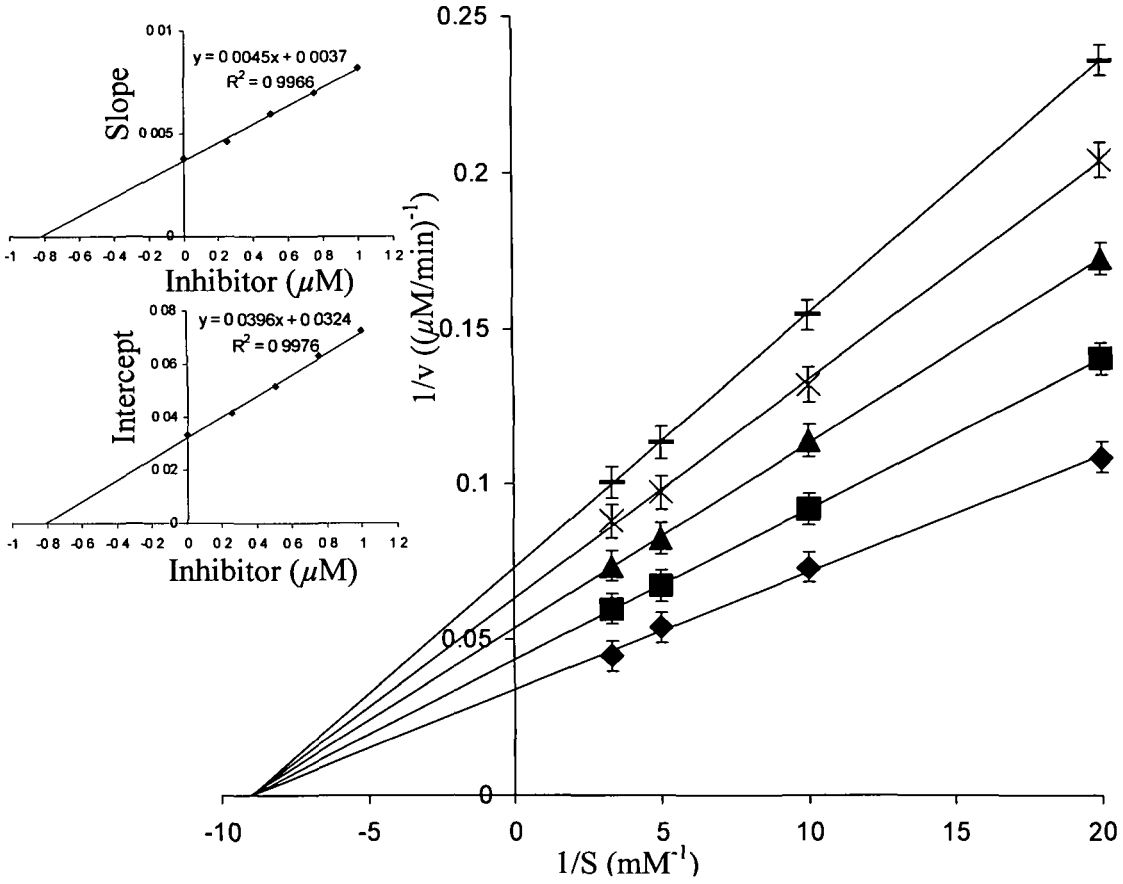


Fig. 7.3 Lineweaver-Burk plots for inhibition of ACP activity in absence and presence of **PAW**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained acetate buffer (0.1 M, pH 4.6) and p-NPP (50-300 μM). The reaction was started by adding ACP (18.38 μg/mL) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 0.25 μM; ▲ 0.50 μM; ∗ 0.75 μM; × 1.00 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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. For polymeric compounds concentrations are on the basis of peroxometal loading.

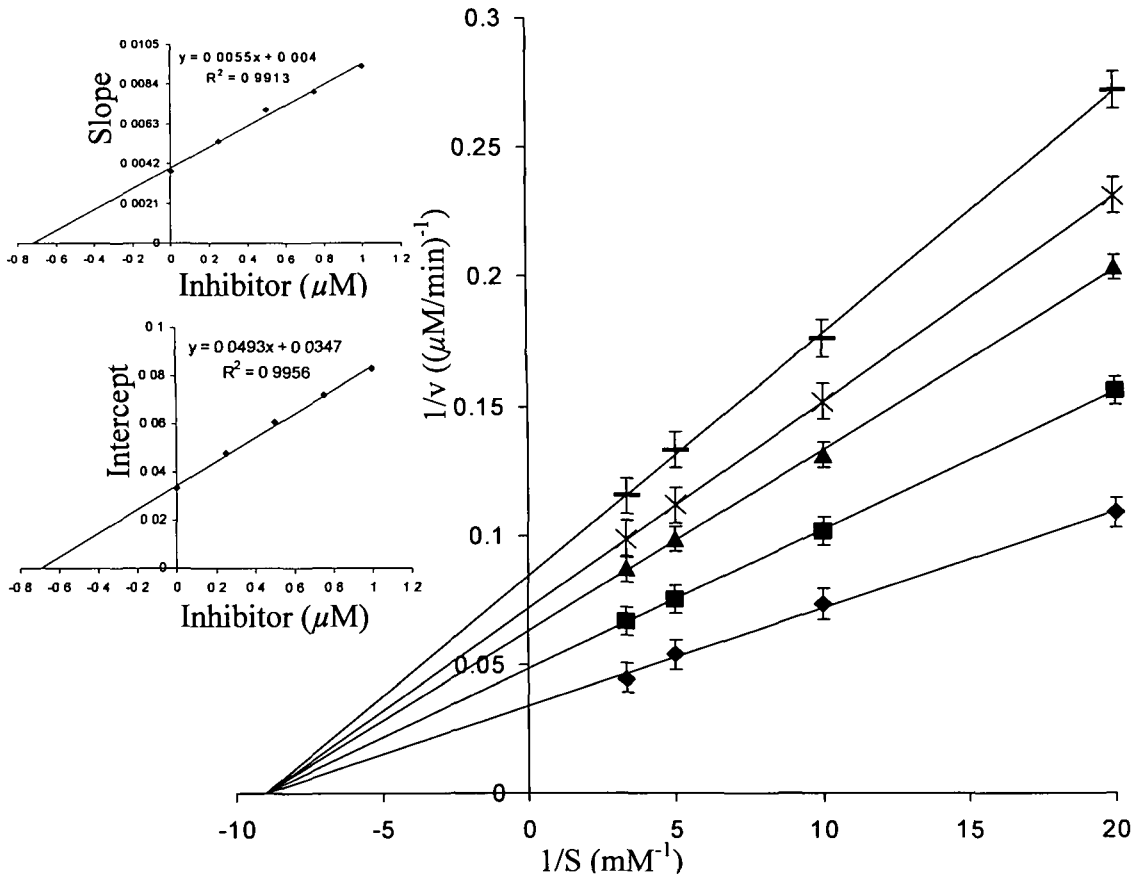


Fig. 7.4 Lineweaver-Burk plots for inhibition of ACP activity in absence and presence of **PMAW**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained acetate buffer (0.1 M, pH 4.6) and p-NPP (50-300 μM). The reaction was started by adding ACP (18.38 μg/mL) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 0.25 μM; ▲ 0.50 μM; ∗ 0.75 μM; × 1.00 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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. For polymeric compounds concentrations are on the basis of peroxometal loading.

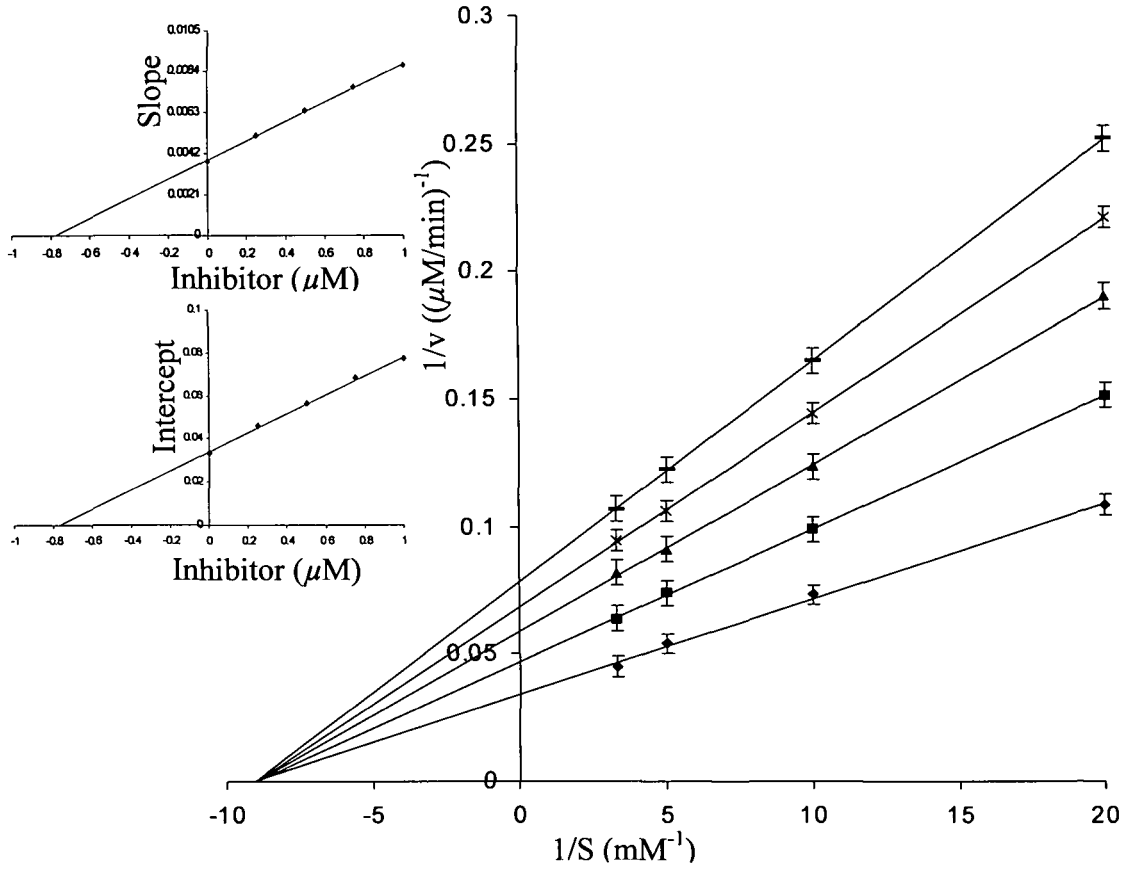


Fig. 7.5 Lineweaver-Burk plots for inhibition of ACP activity in absence and presence of **PAmW**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained acetate buffer (0.1 M, pH 4.6) and p-NPP (50-300 μM). The reaction was started by adding ACP (18.38 μg/mL) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 0.25 μM; ▲ 0.50 μM; ∗ 0.75 μM; × 1.00 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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. For polymeric compounds concentrations are on the basis of peroxometal loading.

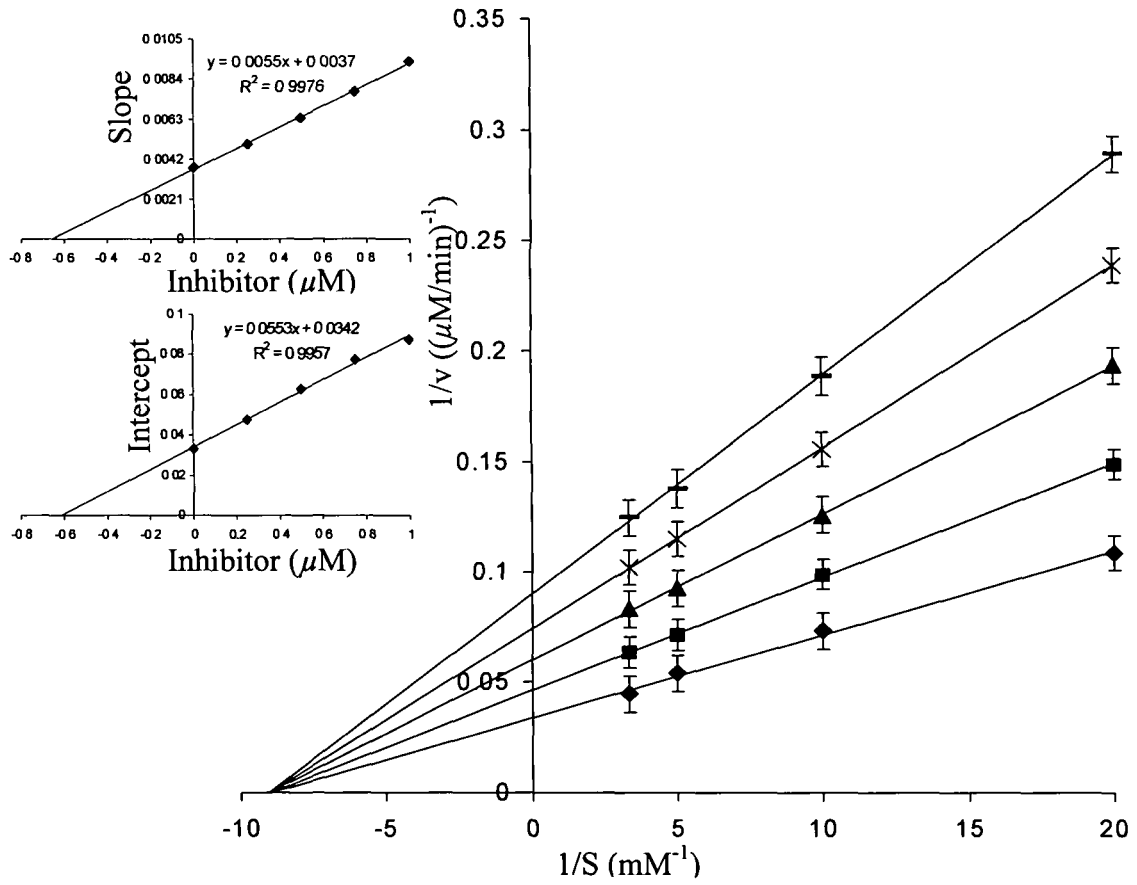


Fig. 7.6 Lineweaver-Burk plots for inhibition of ACP activity in absence and presence of **PSW**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained acetate buffer (0.1 M, pH 4.6) and p-NPP (50-300 μM). The reaction was started by adding ACP (18.38 μg/mL) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 0.25 μM; ▲ 0.50 μM; ⁎ 0.75 μM; × 1.00 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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. For polymeric compounds concentrations are on the basis of peroxometal loading.

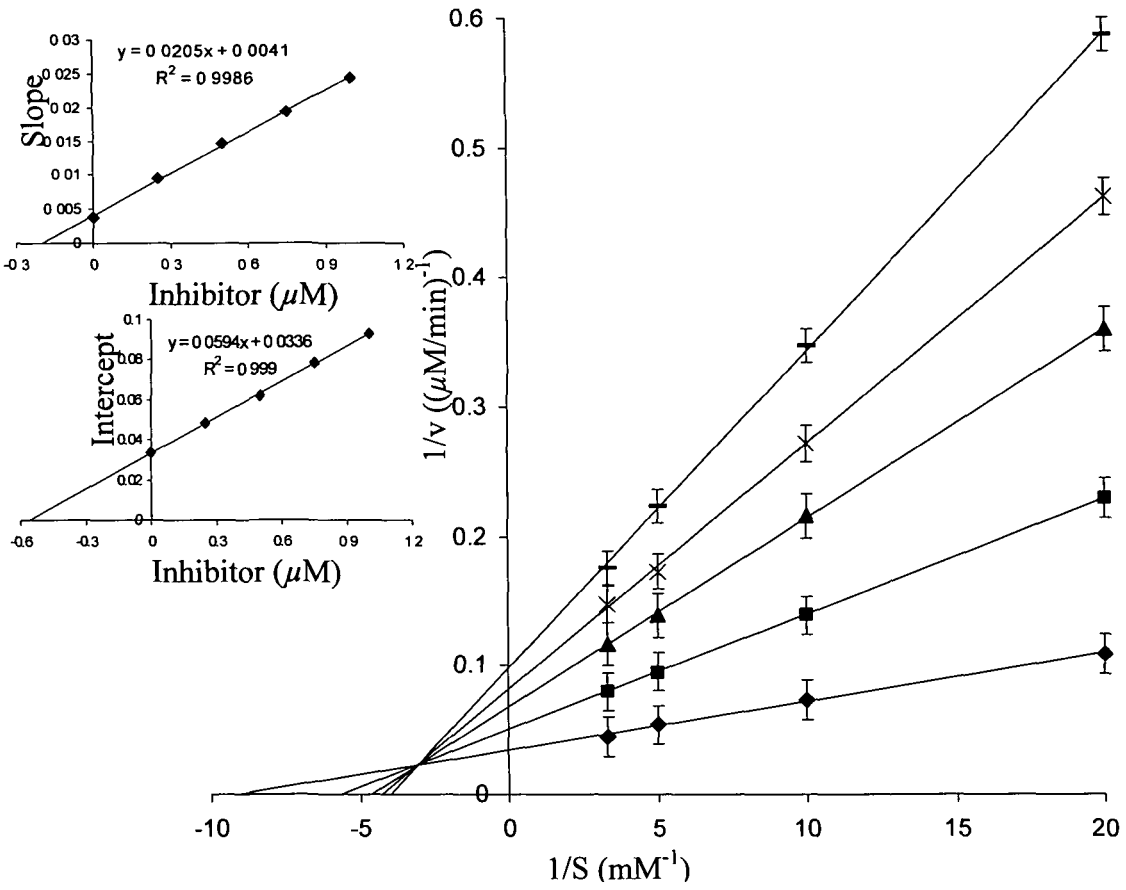


Fig. 7.7 Lineweaver-Burk plots for inhibition of ACP activity in absence and presence of **MWG**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained acetate buffer (0.1 M, pH 4.6) and p-NPP (50-300 μM). The reaction was started by adding ACP (18.38 μg/mL) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 0.25 μM; ▲ 0.50 μM; ⁎ 0.75 μM; × 1.00 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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.

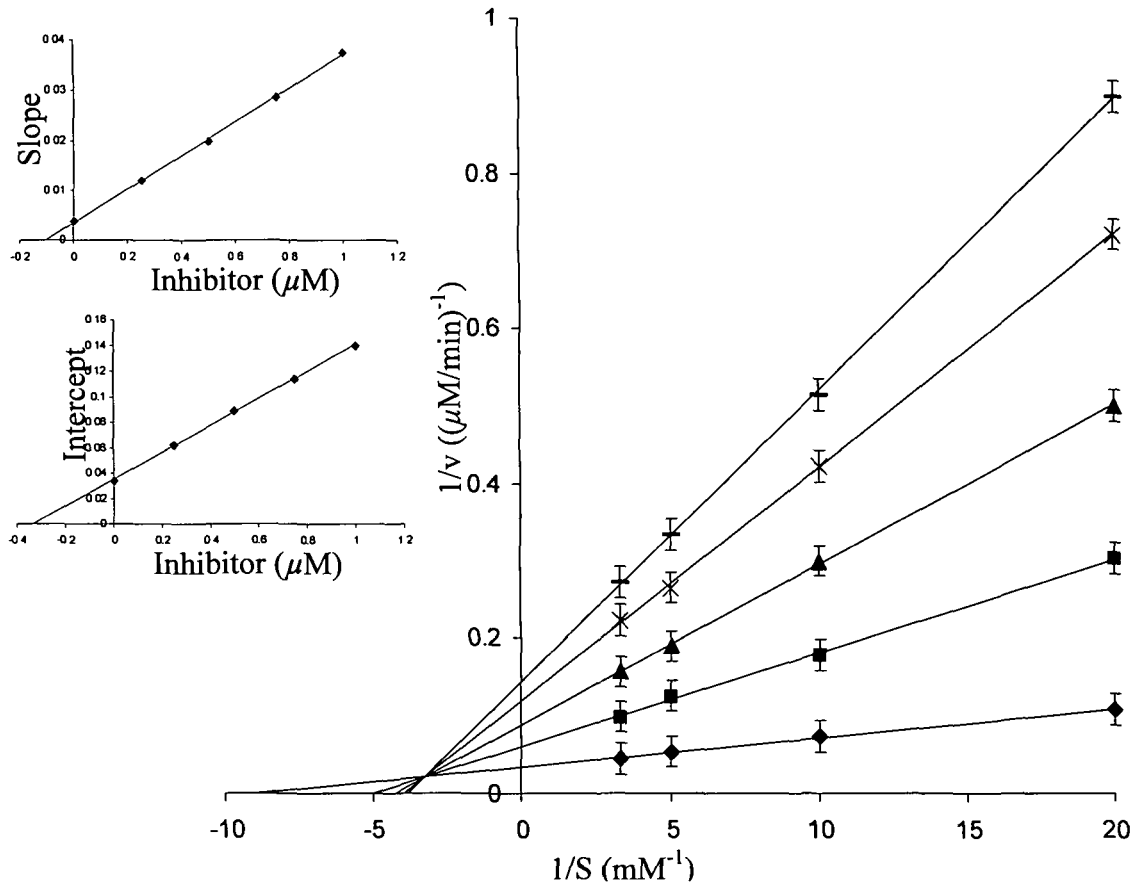


Fig. 7.8 Lineweaver-Burk plots for inhibition of ACP activity in absence and presence of **DWC**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained acetate buffer (0.1 M, pH 4.6) and p-NPP (50-300 μM). The reaction was started by adding ACP (18.38 μg/mL) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 0.25 μM; ▲ 0.50 μM; ⁎ 0.75 μM; × 1.00 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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.

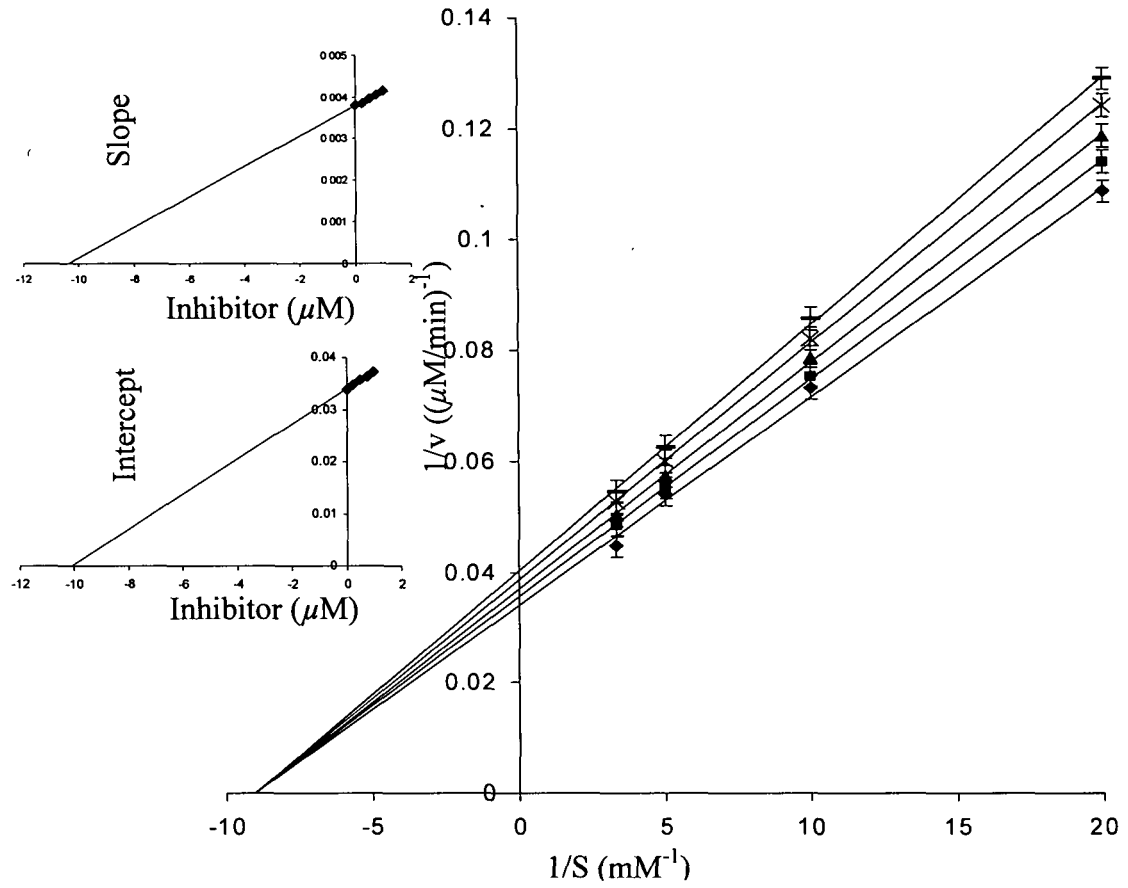


Fig. 7.9 Lineweaver-Burk plots for inhibition of ACP activity in absence and presence of **Na_2WO_4**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained acetate buffer (0.1 M, pH 4.6) and p-NPP (50-300 μM). The reaction was started by adding ACP (18.38 μg/mL) to the reaction solution which was pre-incubated for 5 minutes and the rate of hydrolysis in the presence of ♦ 0 μM; ■ 0.25 μM; ▲ 0.50 μM; ∗ 0.75 μM; × 1.00 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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.

the IC_{50} and inhibitor constants for the two classes of enzymes. These values for ALP was observed to be approximately 25 - 50 orders of magnitude higher than those for ACP which clearly indicated a greater affinity of the complexes for the enzyme binding site of ACP compared to ALP. Presented in Table 7.2 are the kinetic data for inhibition of ALP catalysed hydrolysis of p-NPP by the pW complexes and **Na_2WO_4**. From the Lineweaver-Burk plots Fig. 7.10 – Fig. 7.16 it has been confirmed that polymeric pW compounds are classical non-competitive inhibitors of ALP function. The dinuclear **DWC** is noted to induce mixed-inhibition on the enzyme, as has been observed earlier for **MWG**[46]. Interestingly, our previous findings have also shown the polymer bound pV and pMo to be non-competitive inhibitors of ALP[78]. The kinetic data for the ALP inhibition by the pW compounds indicated the following sequence: **DWC > MWG > PSW > PMAW > PAmW > PAW > Na_2WO_4**. Interestingly, for both the enzymes, ACP and ALP, the free pW complexes were found to be more potent inhibitors in comparison to the polymeric analogues.

Phosphatases are, in general inhibited by oxyanions such as vanadate[17,21,23,39], molybdate and tungstates[49,79]. The competitive inhibition exhibited by these ions on ALP has been attributed to the formation of penta or hexa co-ordinated structures which are described as structural analogues of phosphate[49,79-81]. Gresser and co-workers observed the metal oxyanion inhibition of mammalian acid and alkaline phosphatases to be strictly competitive[49]. In agreement with previous reports we have earlier found that vanadate, molybdate and tungstate inhibit the activity of mammalian ALP in a competitive manner[1,82,83]. While no exception was noted to this observation among the existing literature for ALP inhibition, in case of ACP, type of inhibition induced by these anions was found to be different to that produced by phosphate in certain cases[49,51,72]. For instance, molybdate and tungstate were observed to be non-competitive inhibitor of

bovine spleen purple acid phosphatase[84]. Interestingly, Wang and co-workers reported the molybdate and vanadate to be uncompetitive and non-competitive inhibitors, respectively of the activity of wheat thylakoid membrane ACP[55]. In the present study we observed tungstate to exert non-competitive inhibition on ACP. The information available thus shows that different oxometallates may reveal different mechanistic preferences in these classes of enzymes, in spite of sharing common structural features.

Among the perxometallates, the inhibitory effect of peroxo derivatives of *vanadium on phosphohydrolases have been extensively investigated*[21,23,37,85,86] *although* very little information is available pertaining to similar studies carried out on discreet pMo or pW systems[46-48,78]. Earlier studies suggested that the inhibitory ability of different vanadium derivatives, including peroxovanadates, to modulate ACP or ALP activity depend 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[1,23]. Majority of the synthetic pV compounds tested were competitive inhibitors 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[85,86]. Pertinent here is to mention that our recent investigation on a series of pW[47,48] and pV[46,87] compounds with amino acids and di- and tripeptides as ancillary ligands revealed that such compounds exert mixed-type of inhibition on activity of ALP.

It is known that a competitive inhibitor typically has close structural similarities to the normal substrate for the enzyme. A non-competitive inhibitor usually 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. Since the inhibitor binds at a different site to the substrate, the enzyme may bind to the inhibitor, the substrate or both the inhibitor and the substrate together. The trend emerging out of

Table 7.2 Half-Maximal Inhibitory Concentration (IC_{50}) and Inhibitor Constants (K_i and K_{ii}) Values for pW Compounds and Other Inhibitors Against ALP

Sl. No.	Compound	$IC_{50}(\mu M)$	$K_i(\mu M)$	$K_{ii}(\mu M)$	K_{ii}/K_i	Types of inhibition
1	**PAW**	19.33	17.54	17.13	0.97	Non-competitive
2	**PMAW**	16.12	15.55	15.08	0.96	Non-competitive
3	**PAmW**	17.97	16.23	16.01	0.98	Non-competitive
4	**PSW**	15.95	14.95	14.43	0.96	Non-competitive
5	**MWG**[46]	14.20	14.70	48.20	3.20	Mixed inhibition
6	**DWC**[48]	8.20	6.46	19.10	2.95	Mixed inhibition
7	**Na_2WO_4**[48]	31.68	17.15	---	---	Competitive
8	Free polymer	---	---	---	---	---

Note: The ALP catalyzed rates of hydrolysis of p-NPP at pH 10.0 were determined at 30 °C by measuring A_{405} in a reaction mixture containing ALP (3.3 μg/mL), p-NPP (2 mM) in incubation buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) in the presence of stated concentrations of the inhibitors (Fig. 7.10 - Fig. 7.16). The V_{max} and K_m in absence of inhibitor were found to be 7.9 μM/min and 2.85mM, respectively. For polymeric compounds concentrations are on the basis of peroxometal loading.

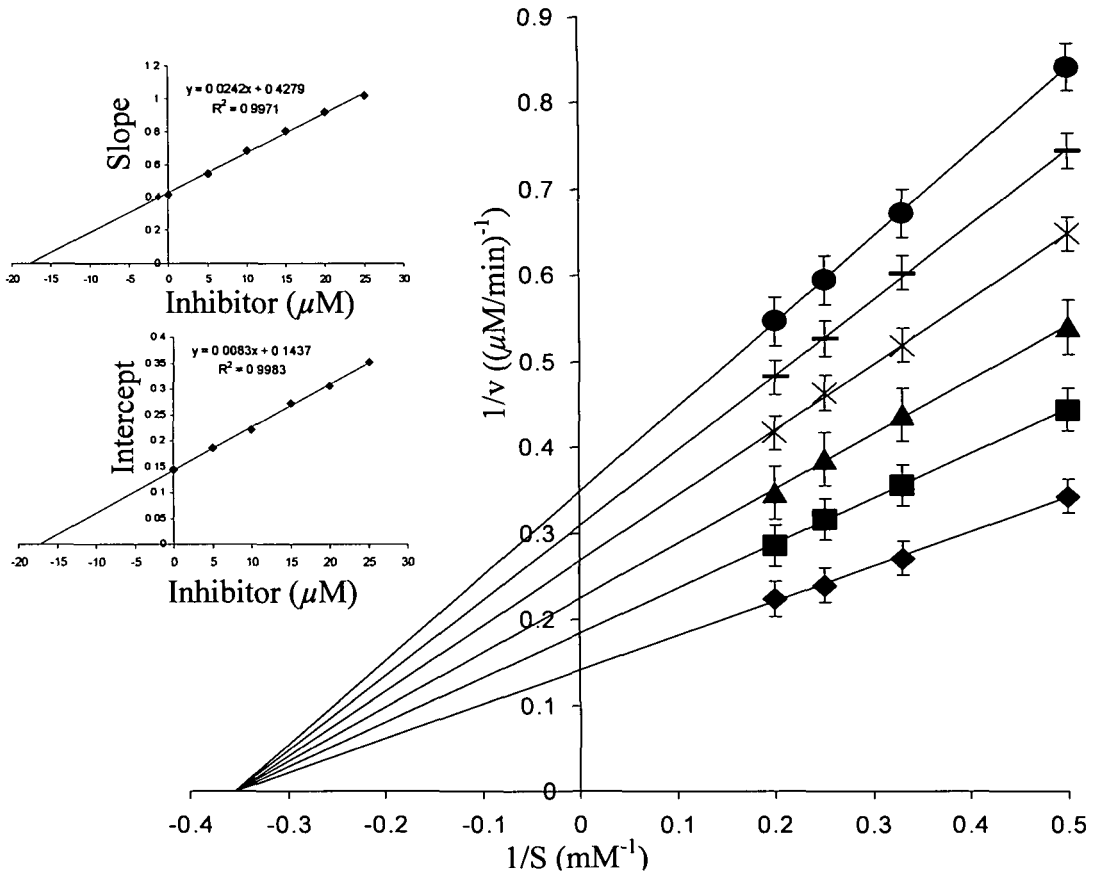


Fig. 7.10 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of **PAW**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and p-NPP (2-5 mM). The reaction was 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; × 15 μM; - 20 μM; ● 25 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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. For polymeric compounds concentrations are on the basis of peroxometal loading.

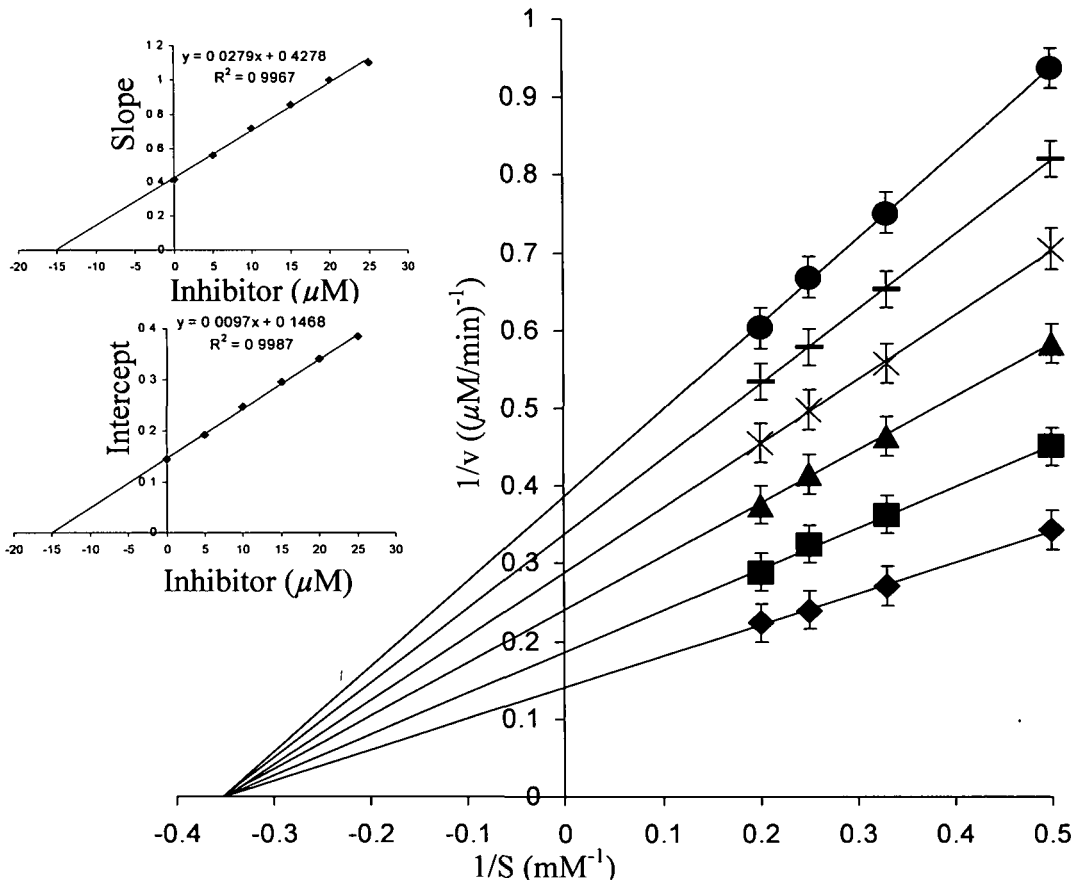


Fig. 7.11 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of **PMAW**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and p-NPP (2-5 mM). The reaction was 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; × 15 μM; - 20 μM; ● 25 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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. For polymeric compounds concentrations are on the basis of peroxometal loading.

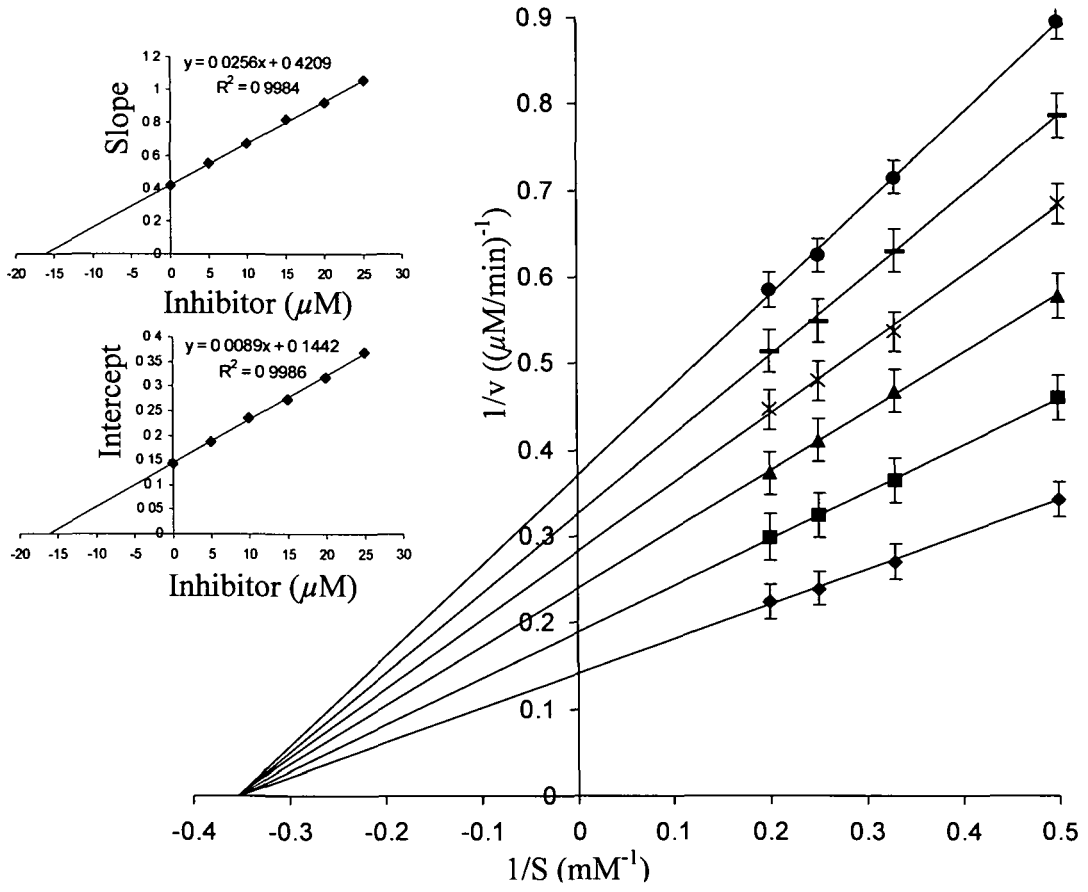


Fig. 7.12 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of **PAmW**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and p-NPP (2-5 mM). The reaction was 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; × 15 μM; - 20 μM; ● 25 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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. For polymeric compounds concentrations are on the basis of peroxometal loading.

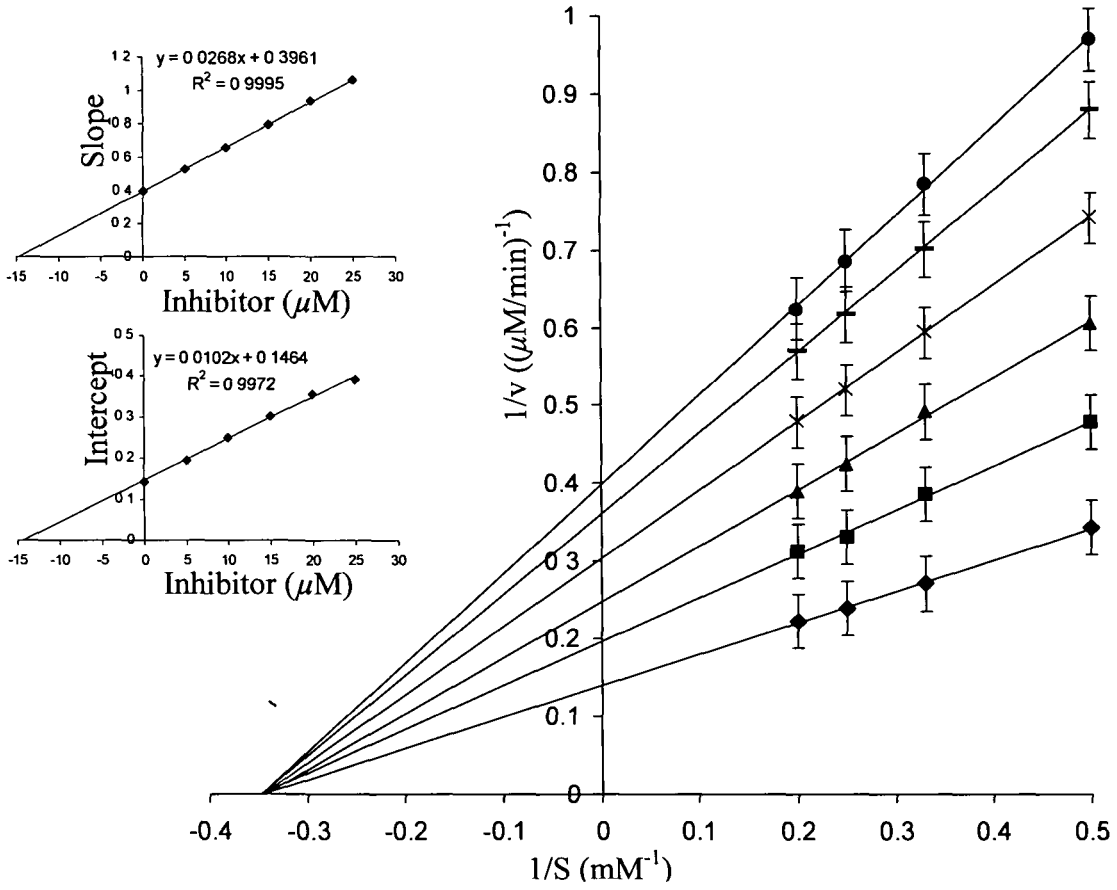


Fig. 7.13 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of **PSW**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and p-NPP (2-5 mM). The reaction was 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; × 15 μM; - 20 μM; ● 25 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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. For polymeric compounds concentrations are on the basis of peroxometal loading.

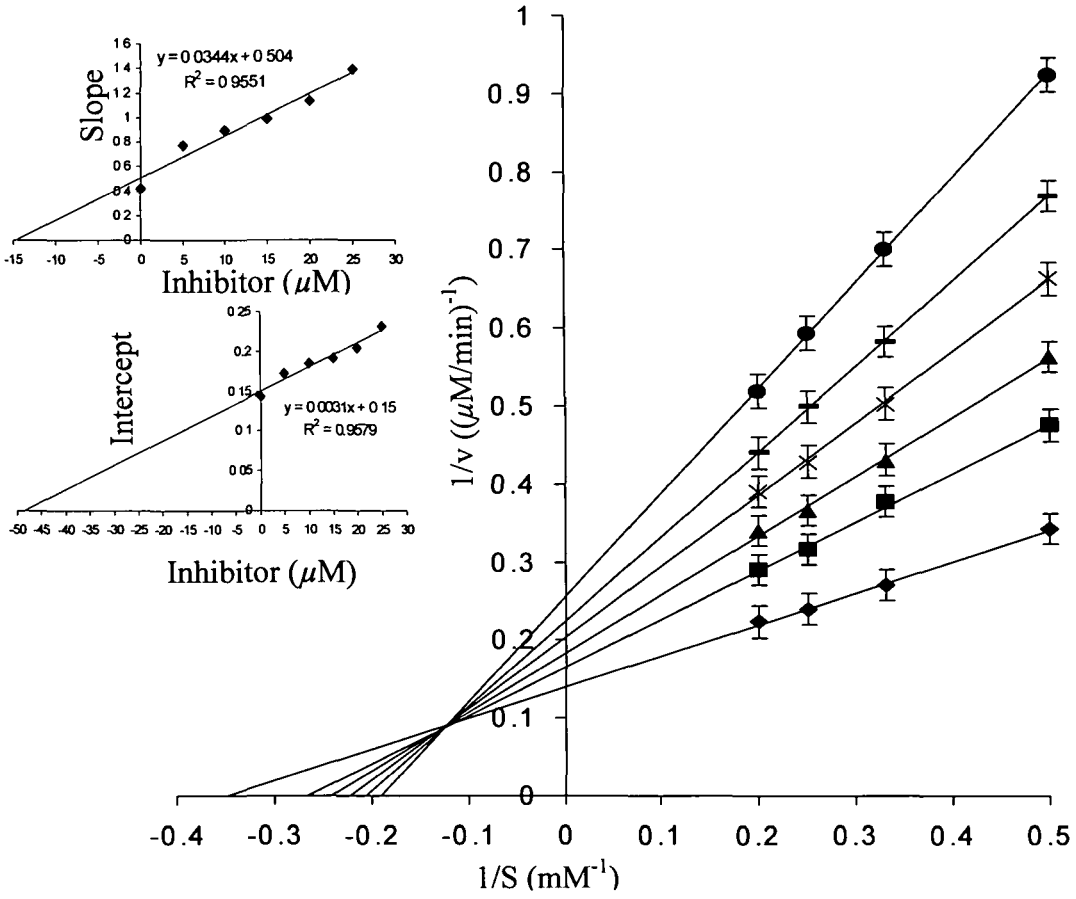


Fig. 7.14 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of **MWG**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and p-NPP (2-5 mM). The reaction was 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; × 15 μM; - 20 μM; ● 25 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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.

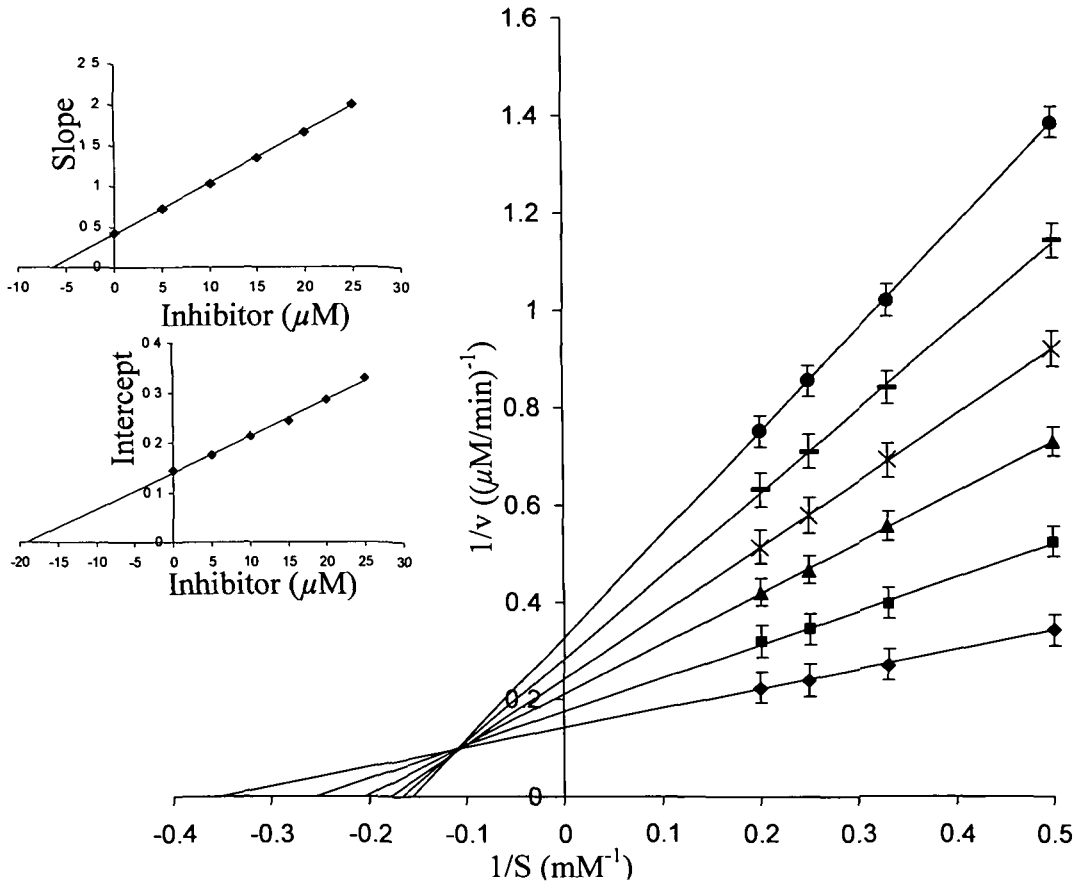


Fig. 7.15 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of **DWC**. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and p-NPP (2-5 mM). The reaction was 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; × 15 μM; - 20 μM; • 25 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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.

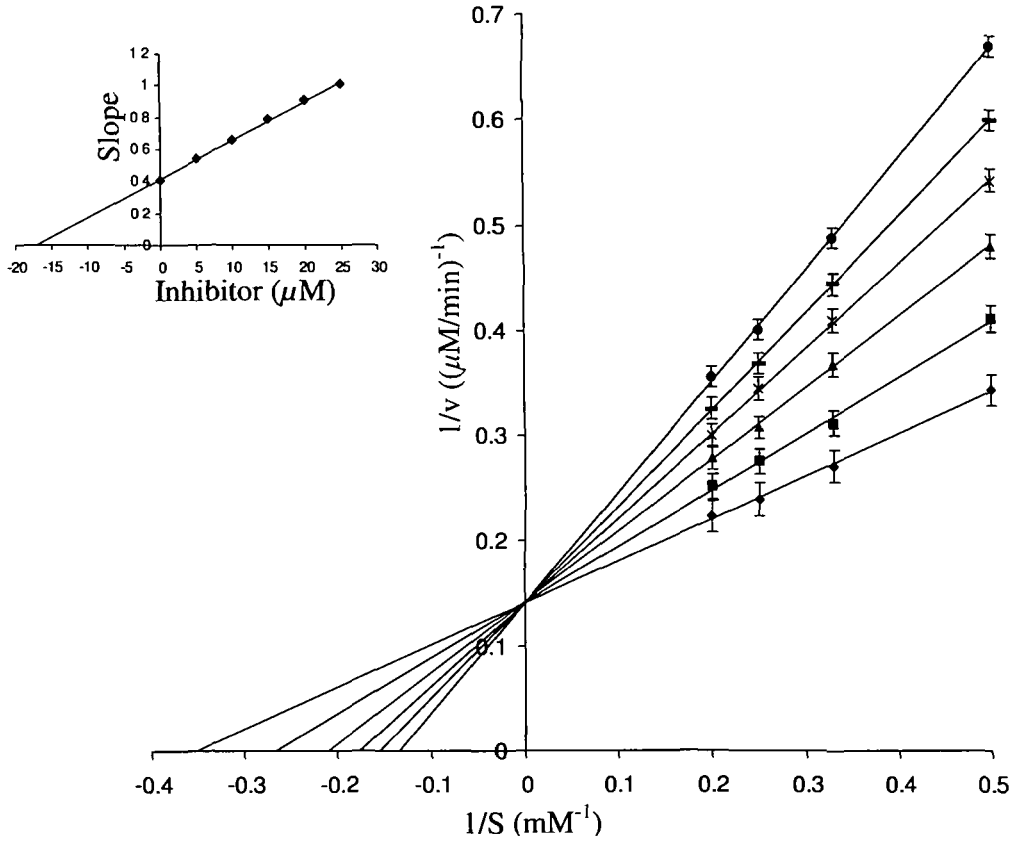


Fig. 7.16 Lineweaver-Burk plots for inhibition of ALP activity in absence and presence of Na_2WO_4. The inset represent secondary plot of initial kinetic data of Lineweaver plot. The reaction mixture contained glycine buffer (25 mM glycine + 2 mM $MgCl_2$, pH 10.0) and p-NPP (2-5 mM). The reaction was 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; × 15 μM; - 20 μM; • 25 μM inhibitors were obtained. The values are expressed as means ± SE from three separate experiments. Inset: (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.

our investigation reveal that the compounds tested can be grouped into two classes on the basis of their effect on the ACP and ALP hydrolysis. The first class comprising of free monomeric or dimeric peroxometallates exhibits mixed inhibition due to the presence of competitive and non-competitive binding sites, whereas the group of polymer bound metal peroxo compounds are classical non-competitive inhibitors of the phosphoproteins. In the present study, factors such as structural analogy with the transition state or phosphate mimicry are unlikely to be the cause responsible for the non-competitive mode of ALP or ACP inhibition exhibited mainly because of their large size. It is relevant to recall that the non-competitive inhibition induced by molybdate and tungstate on bovine spleen purple acid phosphatase was earlier ascribed to the larger size of these ions due to which they bind at a site different from the active site[72].

Due to the complexity of the reaction and species involved we are constrained in drawing any conclusion regarding exact mechanism of inhibition of ALP by the compounds tested. Nevertheless, it is reasonable to expect that oxidant activity of the inhibitor complexes which is also evident from their ability to oxidize sulfide (Chapter 4 and 5)[88], bromide (Chapter 6)[89] as well as GSH[11,47] should be one of the likely causes of the observed inhibitory effect of the compounds on the phosphoproteins. This appears possible keeping in view the literature reports documenting the importance of redox properties of pV compounds in inhibition of protein phosphatases[21,23]. Moreover, it has been suggested that the high oxidative compounds such as molybdate and tungstate probably interact with the Fe^{2+} of the enzyme ACP and oxidize it to ferric and inactivate the enzyme non-competitively[72]. It has been reported that peroxovanadate effectively inhibited the tyrosine phosphatase by oxidizing the critical cysteine residue in catalytic domain of the enzyme[21,90,91]. Correlation has also been found to exist between the GSH oxidizing ability and insulin mimetic activity of peroxo compounds of tungsten and

molybdenum[9]. It may therefore be inferred that the observed enzyme inhibition by the pW compounds originates from their interaction with oxidation prone essential groups such as –SH[38,92]. The presence of such groups in the active centre of the enzymes or in the stabilization of the quaternary structure are essential for enzyme activity[55]. In case of the free pW compounds, both the factors i.e. their transition state analogy as well as oxidant properties are likely to contribute to the observed mixed type of inhibition. However, the mode of action of such macromolecular compounds with complex biomolecules are obviously not simple and many experiments will be needed to deduce the actual mechanism. Although enzyme model systems may not be very effective predictors of *in vivo* activities, nevertheless, the phosphatase inhibition studies can be expected to provide an academic basis for further investigation of the functions of the enzymes in several other reaction courses.

7.3.2 Effect of catalase on the peroxotungsten compounds 3.2-3.5

On incubation with catalase, each of the water soluble polymeric compounds **3.2-3.5** 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. In contrast, addition of catalase to a 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. The rate of degradation of hydrogen peroxide under the effect of catalase was reported to be 430 μM/min from a solution of H_2O_2 of 0.1 mM concentration (Table 7.3)[70]. Thus action of catalase on the newly synthesized compounds (Fig. 7.17) was found to be a slow process compared to H_2O_2. From the Table 7.3 it is evident that the macromolecular complexes are at least 20-30 times weaker as substrate to catalase

compared to H_2O_2. The polymer bound compounds are also 2-3 times more resistant to catalase action relative to free heteroligand diperoxotungsten complex, **MWG (5.1)**.

Total peroxide loss from each of the compound solution having equivalent concentration of co-ordinated peroxide (0.4 mM) was recorded to be *ca.* 0.4 mM, indicating a ratio of 1:2 for peroxide : peroxotungstate which are in excellent agreement with the estimated peroxide content of the compounds.

We have further investigated the degradation of compounds **3.2-3.5** by catalase using UV spectra (Fig. 7.18 and 7.19). It has been observed that the small shoulder like peak in the range of 230-250 nm attributable to diperoxotungsten species[93,94] disappear gradually on incubation of each of the compound (containing 0.4 mM of peroxide) at 30 °C with catalase (40 μg/mL) leaving behind a non specific absorption below 300 nm. The rate of degradation of the compounds obtained from electronic spectral studies agreed well with the values obtained from chemical analysis.

Tested compounds could be arranged in the following sequence of increasing resistance towards degradation under the effect of catalase: H_2O_2 >> **MWG** > **PAW** > **PMAW** > **PSW** > **PAmW**. Thus from the observed trend, it may be inferred that anchoring of pW species to a polymer chain enhances, albeit to different extents, the ability of the co-ordinated peroxo groups of these compounds to resist the action of catalase. The relatively greater resistance of the compounds **3.2-3.5** to the powerful enzyme catalase appears to be a consequence of additional stability imparted to the compounds by the polymeric support.

Table 7.3 Catalase dependent oxygen release from pW compounds

Sl. No.	Compound	Concentration (mg/mL)	Peroxide Content (mM)	Loss of peroxide (μM/min)
1	**PAW (3.2)**	0.122	0.4	20.55
2	**PMAW (3.3)**	0.160	0.4	16.53
3	**PAmW (3.4)**	0.156	0.4	14.41
4	**PSW (3.5)**	0.085	0.4	15.11
5	**MWG (5.1)**		0.4	36.43
6	H_2O_2	----	0.1	430.00

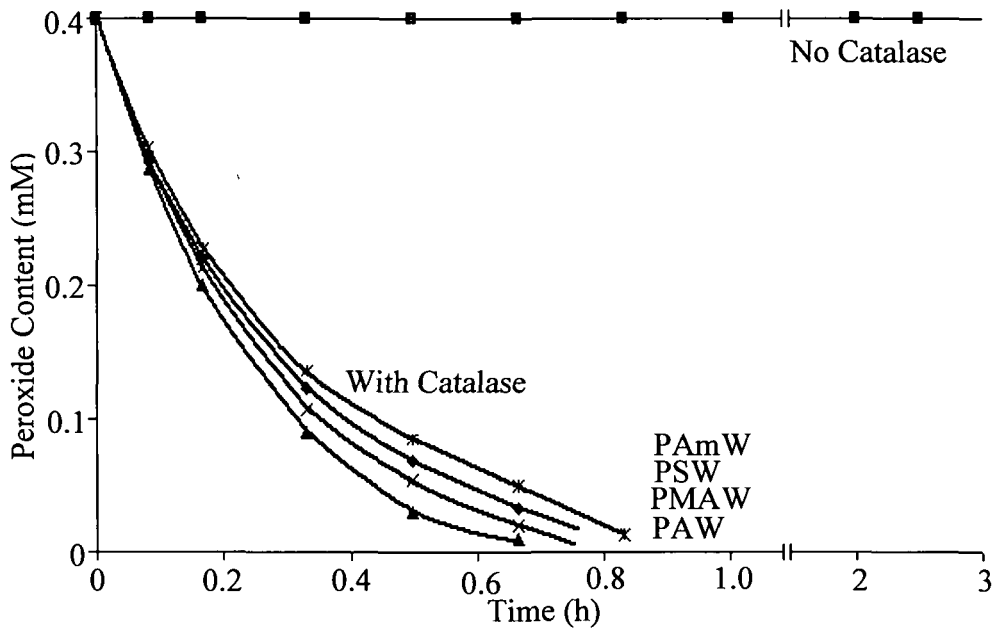


Fig. 7.17 The effect of catalase on **PAW** (▲), **PMAW** (x), **PAmW** (*) and **PSW** (♦). The test solution contained phosphate buffer (50 mM, pH 7.0) and the catalase (40 μg/mL) which was incubated at 30 °C for 5 min. Compounds were then added to the reaction solution and aliquots were drawn at indicated time points and loss in peroxide content was determined. For polymeric compounds concentrations are on the basis of peroxometal loading.

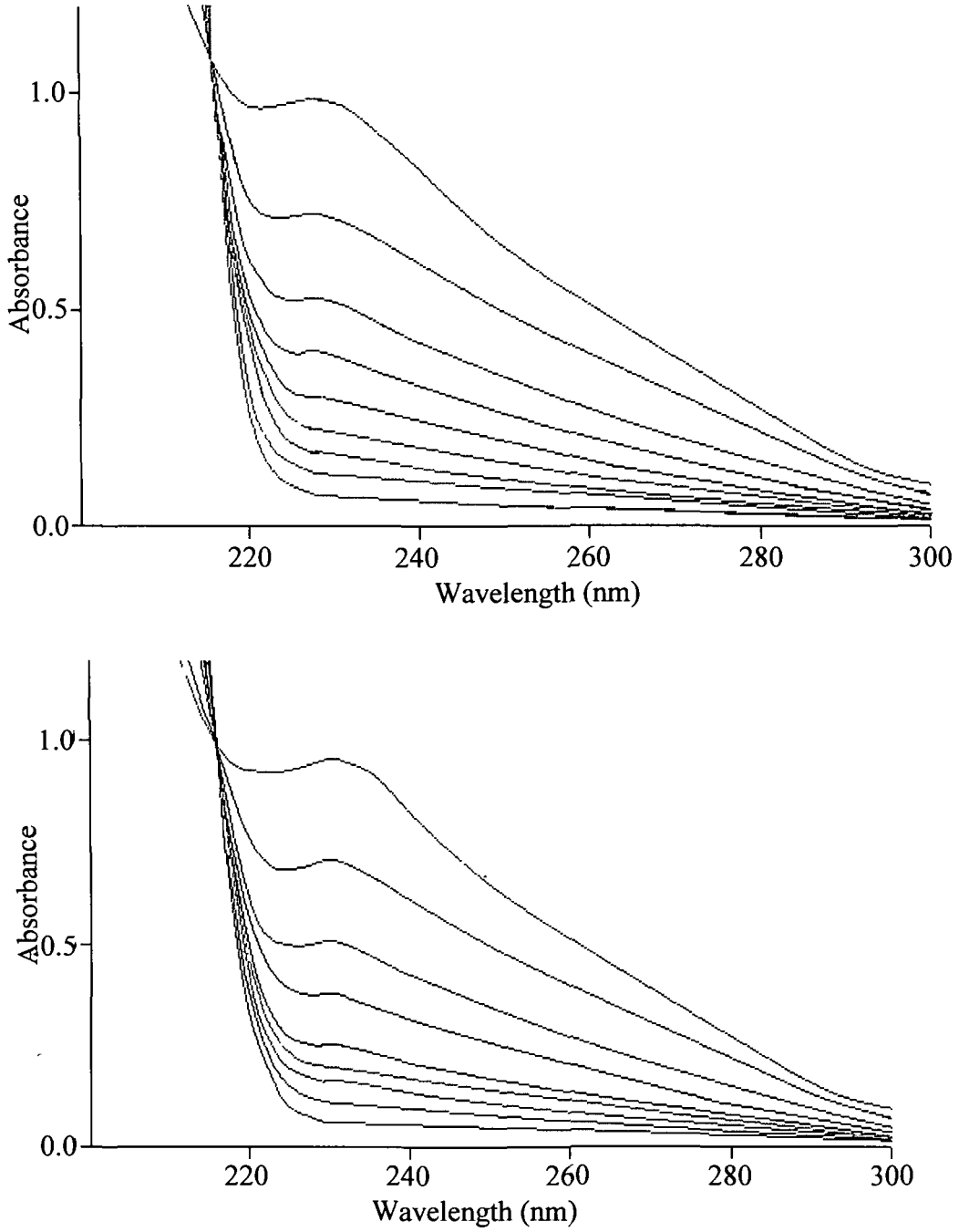


Fig 7.18 Effect of catalase on the compounds. The spectral changes at interval of 10 min on incubation of compound with catalase in solution (a) **PMAW** (A_{235}) and (b) **PSW** (A_{238}). The test solution contained phosphate buffer (50 mM, pH 7.0); compound (0.4 mM of peroxide) and catalase (40 μg/mL) at 30 °C.

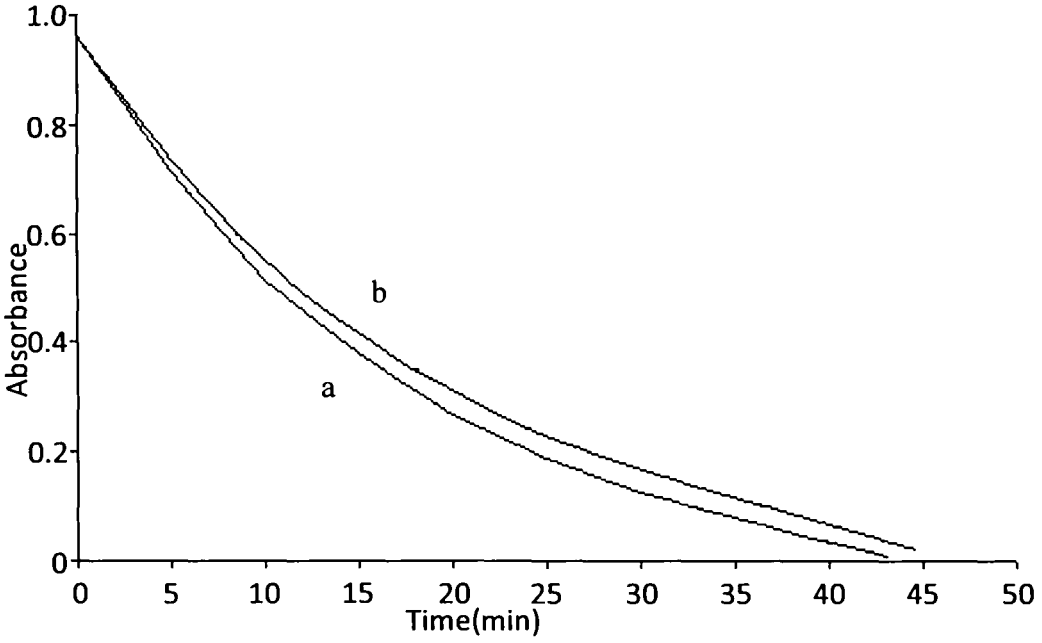


Fig. 7.19 The decrease of absorbance for the compounds (a) **PMAW** (A_{235}) and (b) **PSW** (A_{238}) indicating the rate of degradation of the compounds under the effect of catalase. The reaction mixture contained phosphate buffer (50 mM, pH 7.0); compound (0.4 mM of peroxide) and catalase (40 μg/mL) at 30 °C.

7.4 CONCLUSIONS

To summarize, the present investigation established that peroxotungstates supported on water soluble macromolecules, as well as neat heteroligand pW compounds act as potent inhibitors of two different types of membrane associated phosphohydrolases viz., ALP and ACP. Interestingly, the two classes of complexes examined, anchored and free monomeric or dinuclear peroxotungstates, exhibit distinct mechanistic preferences for phosphatase inhibitory activity. The neat complexes induced mixed type of inhibition of the function of both the model enzymes, whereas the macrocomplexes served as classical non-competitive inhibitors. The pW complexes, irrespective of being supported or free, displayed 25-50 fold greater affinity as inhibitor for ACP than ALP. Taken together, information generated from the present study and results of our previous investigation[46-48,78,87], it is hoped that the supported peroxometallates may serve as excellent selective probes of the non-competitive site of the model systems investigated. Additional remarkable feature of the compounds, likely to be of clinical importance, is their relative resistance to degradation by catalase. There is a search for peroxide derivatives easily formed and stable to degradation that can substitute for H_2O_2 at far lower doses without causing cytotoxicity to the normal cells. It is hoped that the information obtained from the present study will help in identifying the right macroligand environment for peroxo metal derivatives to carry forward for *in vivo* studies.

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List of Publication

1. *New Polymer-Immobilized Peroxotungsten Compound as an Efficient Catalyst for Selective and Mild Oxidation of Sulfides by Hydrogen Peroxide*
   Siva Prasad Das, Jeena Jyoti Boruah, Niharika Sharma and Nashreen S. Islam
   J. Mol. Catal. A: Chem., 356 (2012) 36–45

2. *Selective Oxidation of Organic Sulfides by Mononuclear and Dinuclear Peroxotungsten(VI) Complexes*
   Siva Prasad Das, Jeena Jyoti Boruah, Hiran Chetry and Nashreen S. Islam
   Tetrahedron Lett., 53 (2012) 1163-1168

3. *Polymer-Anchored Peroxo Compounds of Vanadium(V) and Molybdenum(VI): Synthesis, Stability, and Their Activities with Alkaline Phosphatase and Catalase*
   Jeena Jyoti Boruah, Diganta Kalita, Siva Prasad Das, Saurav Paul and Nashreen S. Islam
   Inorg. Chem., 50 (2011) 8046–8062

4. *Vasomodulatory Effect of Novel Peroxovanadate Compounds on Rat Aorta: Role of Rho Kinase and Nitric Oxide/cGMP Pathway*
   Vivek Khanna, Manish Jain, Manoj Kumar Barthwal, Diganta Kalita, Jeena Jyoti Boruah, Siva Prasad Das, Nashreen S. Islam, Tangirala Ramasarma, Madhu Dikshit
   Pharmacol. Res., 64 (2011) 274–282

5. *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., 128 (2009) 200-219

6. *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., 68 (2008) 876-890

7. *Synthesis and Characterization of Peroxotungsten (VI) Complexes Bound to Water Soluble Macromolecules. Their Interaction with Acid and Alkaline Phosphatases*
   Siva Prasad Das, Seshadri Reddy Ankireddy, Jeena Jyoti Boruah and Nashreen S. Islam
   (Communicated)

8. Siva Prasad Das, Jeena Jyoti Boruah, Nashreen S. Islam
   Activity of Polymer-Anchored Peroxotungsten(VI) Compounds as Efficient Oxidant in Mild Oxidative Bromination of Organic Substrates
   (Manuscript Under Preparation)