ABSTRACT

Synthesis and Characterization of Heteroleptic Peroxo Compounds of Niobium(V). Their Applications as Oxidation Catalysts and Enzyme Inhibitors

ABSTRACT

The thesis deals with the synthesis and characterization of a variety of heteroleptic peroxoniobium complexes, including peroxoniobates (pNb) anchored to water soluble polymers. The thesis also presents an account of the results of investigation on activities of pNb compounds as (i) enzyme inhibitors, (ii) versatile catalysts for organic oxidations under ecologically acceptable reaction condition. The contents of the thesis have been distributed over eight chapters.

Chapter 1 gives a general introduction along with a literature review, describing the outline and scope of the present investigation in the background of known chemistry of niobium, with a special emphasis on general aspects and recent advances in the field of peroxo niobium chemistry. The importance of immobilization of metal complexes on linear water soluble polymers from the chemical as well as biological perspectives has also been highlighted in this chapter. Attention is being drawn to the paucity of information on polymer supported peroxometallates and to the fact that potential of discreet pNb compounds as biologically active agents remains relatively unexplored despite the knowledge that niobium and its compounds display favourable bio-relevant characteristics.

Chapter 2 describes the details of materials used, methods of elemental analysis, techniques employed for the characterization and reactivity study of the synthesized compounds.

Chapter 3 illustrates the synthesis of a pair of novel peroxoniobium complexes anchored to water soluble polymer matrices such as poly(acrylate) and poly(styrene sulfonate) of the type,$[Nb_2(O_2)_6(carboxylate)_2]$-PA [PA = poly(sodium acrylate) **(PANb)** (**3.1**)] and $[Nb(O_2)_3(sulfonate)_2]$-PSS [PSS = poly(sodium styrene sulfonate) **(PSSNb)** (**3.2**)].The macro complexes were obtained by methodology involving the reaction of the pre formed $Na_3[Nb(O_2)_4]\cdot 13H_2O$ **(NaNb)** with the respective macro-ligand in presence of excess H_2O_2 maintaining the pH at *ca.* 5. Synthesis of the compounds was found to be sensitive to reaction temperature and concentration of the reactants, in addition to pH.

The pNb macro complexes were characterized by elemental analysis (CHN, ICP and energy-dispersive X-ray spectroscopy), spectral studies (FTIR, Raman, ^{13}C and ^{93}Nb NMR studies), thermogravimetric analysis (TGA) as well as SEM studies. The viability of structures of **PANb** (**3.1**) and **PSSNb** (**3.2**) was studied by density functional theory (DFT) method. The structure of **PANb** (**3.1**) comprises of Nb(V) atoms with three side-on bound peroxo groups, bonded to the polymer chain *via* its pendant carboxylate groups in a bridged bidentate manner. The complex **PSSNb** (**3.2**) includes Nb linked to two unidentate sulfonate groups of polymer side chain, with three [η^2]-peroxo moieties completing eight coordination around Nb(V) central atom.

Chapter 4 focuses on synthesis of a series of heretofore unreported heteroleptic triperoxoniobium complexes with biogenic species as co-ligands of the type, $Na_2[Nb(O_2)_3L]$ [L = alaninato (**NbAla) (4.1)** or valinato (**NbVal) (4.2**)], $Na_2[Nb(O_2)_3(arg)]\cdot 2H_2O$ [arg = arginate **(NbA**) (**4.3**)], and $Na_2[Nb(O_2)_3(nic)(H_2O)]\cdot H_2O$ [nic = nicotinate **(NbN)** (**4.4**)]. The synthesized compounds were comprehensively characterized by elemental analysis, spectral studies (FTIR, Raman, ^1H NMR, ^{13}C NMR and ^{93}Nb NMR), EDX analysis and TGA-DTG analysis. The crystal structure of potassium salt of the tetraperoxoniobate complex, $K_3[Nb(O_2)_4]$ **(KNb)** determined by single crystal X-ray analysis is also reported in this Chapter. The structures of the heteroleptic pNb complexes were studied by density functional theory (DFT) method. Theoretically obtained IR and Raman frequencies calculated for the optimized geometries correlated well with the respective experimentally determined data.

In **Chapter 5** we have reported the activity of monomeric peroxoniobium (pNb) complexes, **4.3** and **4.4** along with pNb macrocomplexes, **3.1** and **3.2** as highly efficient homogeneous catalysts, for the selective oxidation of a variety of thioethers to the corresponding sulfoxide or sulfone with 30% aqueous H_2O_2 in water. The reactions proceeded under mild conditions to afford the resulting products in excellent yields with good TON or TOF. The catalysts can be recycled up to six reaction cycles without losing their activity or selectivity. The oxidation is chemoselective for sulfides or sulfoxides leaving the C=C or alcoholic moiety unaffected. The developed catalytic procedure thus provide an eco compatible alternative as it involves water as solvent, H_2O_2 as green oxidant and is completely free from organic solvents, or co-catalysts.

Chapter 6 provides an account of our findings of investigation on some biologically significant features of the synthesised complexes, including the macro complexes, such as their hydrolytic stability, cytotoxicity and their interaction with the enzyme catalase. The stability of the compounds, homoleptic tetraperoxoniobate **NaNb** and **KNb,** monomeric pNb complexes **4.1-4.4** and macrocomplexes **3.1**, **3.2** in solution of a wide range of pH values ranging from 1.2 to 8.0 has been examined. The results revealed that the compounds retain their structural integrity in acidic as well as higher pH. The pNb compounds displayed significant resistance to degradation under the effect of catalase, the ubiquitous reactive oxygen mopping enzyme, relative to its natural substrate, H_2O_2. The difference in rates of degradation between H_2O_2 and the pNb species indicates that the compounds are at least 20-60 fold weaker as substrate to the enzyme, with respect to H_2O_2.

The cytotoxicity of the developed pNb compounds towards Raw 264.7 murine macrophage cells was assessed by determining cell viability employing MTT assay. No significant effect on cell viability was observed even at relatively higher compound concentration (200 μM) reflecting their minimal toxicity to the cells.

Presented in **Chapter 7**, are the results of a study on activity of peroxoniobium (pNb) complexes belonging to three different classes *viz.*, (i) the homoleptic tetraperoxoniobate compounds, $Na_3[Nb(O_2)_4]\cdot 13H_2O$ (**NaNb**), $K_3[Nb(O_2)_4]$ **(KNb)**, (ii) heteroleptic compounds, $Na_2[Nb(O_2)_3L]$ [L = alaninato (**NbAla) (4.1)** or valinato (**NbVal) (4.2**)], $Na_2[Nb(O_2)_3(arg)]\cdot 2H_2O$ [arg = arginate **(NbA**) (**4.3**)], $Na_2[Nb(O_2)_3(nic)(H_2O)]\cdot H_2O$ [nic = nicotinate **(NbN)** (**4.4)** and (iii) the macro complexes,$Nb_2(O_2)_6(carboxylate)_2$]-PA [PA = poly(sodium acrylate) **(PANb)** (**3.1**)], $[Nb(O_2)_3(sulfonate)_2]$-PSS [PSS = poly(sodium styrene sulfonate) **(PSSNb)** (**3.2**)] as inhibitors of membrane associated phosphohydrolase, acid phosphatase (ACP). Employing wheat thylakoid acid phosphatase as a model enzyme it has been demonstrated for the first time that peroxoniobium derivatives serve as active inhibitors of phosphatase with IC_{50} values varying within the range of < 9 μM. The results of detailed kinetic analysis revealed that the monomeric pNb compounds, irrespective of the nature of their co-ordination environment, exert mixed type of inhibition on ACP activity whereas, each of the macromolecular complexes, *viz.*, **PANb** and **PSSNb**, behave as classical non-competitive inhibitors of ACP ($K_i = K_{ii}$).

Chapter 8 elaborates the identification of free monomeric as well as polymer immobilized peroxo complexes of niobium as a novel class of potent inhibitors of the enzyme calcineurin. Calcineurin (CN) is a calmodulin binding serine/threonine phosphatase which plays a crucial role in numerous mammalian signal transduction pathways. Apart from their clinical significance, CN inhibitors are useful as valuable tool for basic research. The *in vitro* effect of the complexes *viz.*, $Na_3[Nb(O_2)_4]\cdot 13H_2O$ (**NaNb**) and the macro complex, $[Nb_2(O_2)_6(carboxylate)_2]$-PA (**PANb**), on calmodulin mediated dephosphorylation activity of CN was investigated using two different types of substrates *viz.*, a physiological substrate of calcineurin, RII-phosphopeptide and a non-protein substrate *p*-nitrophenyl phosphate *(p*-NPP). The peroxo metal derivatives were observed to be nearly 6 fold more potent as inhibitors (IC_{50} = 5.5 – 7.5 μM) than hydrogen peroxide (IC_{50} = 32.5 μM), a significant cellular oxidant. Other important findings of the study, as revealed by enzyme kinetic analysis, are the uncompetitive nature of the inhibition of calcineurin by the compounds with K_{iu} values 2.4 μM for **NaNb**, 2.7 μM for **PANb** and 11.3 μM for H_2O_2, suggesting the formation of an enzyme-inhibitor-substrate complex during the course of inhibition.

The major part of the results of the studies described in **Chapters 3-8** has been published and the rest is under communication.