

CHAPTER 2

Chapter 2**Materials and Methods**

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This chapter describes various analytical and physicochemical techniques employed for the characterization of the synthesized peroxoniobium complexes. Procedural details about the synthesis of the complexes and their activity studies are discussed in their respective chapters.

2.1 Chemicals

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

Acetone, acetonitrile, methanol, ethylacetate, petroleum ether, diethyl ether, dichloromethane, silica gel (60-120 mesh), 30% hydrogen peroxide, ammonium acetate (RANKEM), L-alanine, L-valine, L-arginine, nicotinic acid (CDH, New Delhi, India), sodium hydroxide, sodium sulfate, methanol, acetonitrile (E. Merck, India), poly(sodium acrylate) ($M_w = 2100$) (Fluka), poly(sodium 4-styrene sulfonate) ($M_w = 200000$), acid phosphatase from wheat thylakoid membrane (ACP), calcineurin cellular activity assay kit (Calbiochem) was purchased from Merck, India, catalase, *p*-nitrophenyl phosphate (*p*-NPP), niobium pentoxide, N-benzoyl-N-phenylhydroxylamine, methyl phenyl sulfide (MPS), methyl *p*-tolylsulfide (MpTS), ethyl phenyl sulfide (EPS), dimethyl sulfide (DMS), dibutylsulfide (DBS), phenylvinylsulfide (PVS), 2-(phenylthio)ethanol (PTE), dihexylsulfide (DHS), diphenylsulfide (DPS), allyl phenyl sulfide (APS), tween-20 and malachite green were obtained from Sigma-Aldrich Chemical Company, Milwaukee, WI, USA and sodium thiosulfate, potassium hydrogen phosphate, potassium dihydrogen phosphate and $MgCl_2$ (SD Fine Chemicals, Mumbai, India). The precursor for pNb complex synthesis, $Na_3[Nb(O_2)_4] \cdot 13H_2O$ (**NaNb**), was synthesized according to a previously reported procedure [1].

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

2.2 Elemental analyses

2.2.1 Niobium

2.2.1.1 Gravimetry [2]

Niobium was estimated by precipitation as $NbO(C_{13}H_{10}NO_2)_3$ by adopting the following procedure [2]. An accurately weighed amount of the synthesized compounds were dissolved in 20 mL distilled water in a 250 mL beaker and then pH 6 was maintained for the solution with ammonium acetate. To the mixture, N-benzoyl-N-phenylhydroxylamine reagent was added followed by heating up to boiling. A buff-coloured precipitate was observed and the precipitate was then filtered through a

constant-weighted sintered glass crucible and washed with hot water until free from the reagent. After washing, it was dried to constant weight at 110 °C.

2.2.1.2 EDX analysis, inductively coupled plasma optical emission spectrophotometer (ICP-OES) and atomic absorption spectroscopy (AAS)

Niobium content was also determined by using Energy Dispersive X-Ray (EDX) analysis, inductively coupled plasma optical emission spectrophotometer and atomic absorption spectroscopy (AAS).

2.2.2 Peroxide [3-6]

2.2.2.1 Permanganometry [3]

An accurately weighed amount of a peroxoniobium(V) compound was dissolved to a freshly prepared solution of 7 N sulfuric acid containing *ca.* 4 g of boric acid. To prevent any loss of active oxygen from the solution, boric acid was used which will form perboric acid. The resulting solution was then titrated with a standard potassium permanganate solution. This method is appropriate for determination of peroxide content in peroxoniobium(V) compounds.

$$1 \text{ mL of } 1\text{N KMnO}_4 = 0.01701 \text{ g of H}_2\text{O}_2$$

2.2.2.2 Iodometry [4,5]

An accurately weighed amount of the peroxoniobium(V) compound was dissolved to a freshly prepared 2 N sulfuric acid solution containing an appropriate amount of potassium iodide (~1 g in 100 mL) and 0.5 g of sodium bicarbonate. The mixture was kept in CO₂ atmosphere in the dark for *ca.* 15 min. The amount of liberated iodine was titrated against standard sodium thiosulfate solution until it turned into pale yellow followed by addition of few drops (1-5 mL) of 1% starch solution, and the solution was turned into deep purple colour. Subsequently, it was titrated until the solution became colourless and the final reading was observed.

$$1 \text{ mL of } 1\text{N Na}_2\text{S}_2\text{O}_3 = 0.01701 \text{ g of H}_2\text{O}_2$$

The total amount of peroxide present in the compound can be determined by this method.

2.2.2.3 By standard Ce(IV) solution [6]

An accurately weighed amount of a peroxoniobate(V) compound was added to 0.7 N sulfuric acid solution in the presence of an excess of boric acid. Titration was carried out with standard Ce(IV) sulfate solution to estimate the peroxide percentage of the compound.

2.2.3 Carbon, hydrogen and nitrogen

The carbon, hydrogen and nitrogen percentage present in the compounds were analyzed by Perkin Elmer 2400 series II CHN elemental analyzer at the Department of Chemical Sciences, Tezpur University.

Carbon and nitrogen contents were also determined from EDX analysis.

2.2.4 Sulfur

The sulfur content in the compounds was determined by EDX analysis.

2.2.5 Sodium

Sodium content in the compounds was determined by EDX analysis and ionometer.

2.3 Physical and spectroscopic measurements

2.3.1 pH measurement

The reaction solutions pH, whenever required, was measured by using an Orion VERSARSTAR pH meter and also by E. Merck Universal indicator pH 0-14 paper.

2.3.2 Electronic spectra

The absorption spectra in the UV-Vis region were recorded using 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_{405} , A_{655} at the wavelengths indicated.

2.3.3 Infrared (IR) and Raman spectra

The infrared (IR) spectra were recorded in KBr pellets using a Perkin-Elmer spectrum 100 FTIR spectrophotometer in the range 4000-400 cm^{-1} . Raman spectra of the compounds were recorded using a Renishaw InVia Raman microscope equipped with an argon ion laser with an excitation wavelength of 514 nm and a laser maximum output power of 20 mW. The measurement parameters were 10 s of exposure time, 1 accumulation, laser power 10% of the output power, 50 \times objective and the spectral resolution was set to 0.3 cm^{-1} .

2.3.4 Surface morphology analysis by Scanning Electron Microscope (SEM)

The JEOL JSM-6390LV Scanning Electron Micrograph attached with energy dispersive X-ray detector was used to analyze the surface morphology of the samples. The samples were prepared on SEM mounts with carbon tape which was coated with a thin layer of evaporated platinum. Scanning was done at 1-20 μm range and images were taken at a magnification of 15-20 kV. INCA software was used to obtain the data. The standardization of the data analysis is an integral part of SEM-EDX instrument employed.

2.3.5 Atomic Absorption Spectroscopy (AAS)

Atomic Absorption Spectrometry was carried out with a Thermo iCE 3000 series Atomic absorption spectrophotometer model analyst 200.

2.3.6 ^1H NMR spectra

The JEOL JNM-ECS400 spectrophotometer was used to record ^1H NMR spectra using deuterated solvents. The chemical shifts are referenced with respect to TMS = δ 0 ppm. The values are given in ppm and s, d, m and br abbreviations are used to represent the singlet, doublet, multiplet and broad absorption signals respectively in the ^1H -NMR spectrum at 400 MHz.

2.3.7 ^{13}C NMR spectra

The NMR instrument JEOL JNM-ECS 400 spectrometer was used to record ^{13}C NMR spectra for all the peroxoniobium complexes at carbon frequency 100.5 MHz.

For all the water soluble peroxoniobium complexes, the ^{13}C NMR spectra were recorded at a carbon frequency of 100.5 MHz, 131 072 X-resolution points, number of scans 8000-20000, 1.04 s acquisition time and 2.0 s relaxation delay with the ^1H NMR decoupling method in D_2O as solvent. The NMR spectra of organic sulfoxides and sulfones were recorded in CDCl_3 .

2.3.8 ^{93}Nb NMR spectra

For ^{93}Nb NMR spectra: a niobium frequency of 97.85 MHz, 32,768 X-resolution points, 1024 number of scans, 83 ms of acquisition time and 90° pulse length. The ^{93}Nb NMR chemical shifts were referenced to an external saturated solution of NbCl_5 in CD_3CN .

2.3.9 GC analysis

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

2.3.10 HPLC analysis

HPLC analysis were done by using a Waters Tm 2487 dual k detector and assayed at fixed wavelengths using a C_{18} , column (Nova-Pak C_{18} , 3.9 x 150 mm, Waters) with HPLC grade ethyl acetate and n-hexane solvent system.

2.3.11 Thermogravimetric analysis

Thermogravimetric analysis was carried out with the SHIMADZU TGA-50 and Perkin-Elmer STA 6000 system using aluminium pan (heating rate = $10^\circ\text{C}/\text{min}$) under an N_2 atmosphere.

2.3.12 Melting point determination

Melting points were determined in open capillary tubes using a Büchi Melting Point B-540 apparatus.

2.3.13 Magnetic susceptibility

Magnetic susceptibilities measurement of the complexes was carried out by the Gouy Method [5], using Hg [Co(NCS)₄] as the calibrant with Magnetometer (Model EMU 50).

2.4.1 Computational calculation

Density functional theory (DFT) method was employed for computational calculations. For monomeric pNb compounds, the density functional theory (DFT) [7] calculations were performed using the Gaussian09 programme [8] at the B3LYP/LANL2DZ level of theory. The ground-state geometries of the two niobium complexes were obtained in the gas phase and the minima of the optimized structures were verified by the absence of imaginary frequencies. For polymer anchored pNb macrocomplexes, DFT calculations were performed on the model complexes using the BLYP functional and DNP basis set as implemented in the DMol³ program [9,10].

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