CHAPTER II

EXPERIMENTAL TECHNIQUE
11.1 SAMPLE PREPARATION AND DATA COLLECTION

X-ray absorption data were collected on biomolecules and several copper compounds which serve as models. This section describes the preparation of the samples and outlines the conditions under which the X-ray absorption spectra were measured.

11.2 PREPARATION OF MATERIALS

11.2.1 PREPARATION OF MODEL COPPER COMPOUNDS

$\text{Cu}_2\text{O}$, $\text{CuO}$, $\text{CuBr}$, $\text{CuBr}_2$, $\text{CuCl}$, $\text{CuI}$, $\text{CuCN}$, $\text{CuF}_2$, copper phthalocyanine, $[\text{C}_32\text{H}_{16}\text{CuN}_4]$, and cupric acetylacetonate, $[\text{Cu(C}_5\text{H}_7\text{O}_2)_2]$ were obtained from M/S. Johnson Matthey GmbH-Alfa Products, Karlsruhe, Germany. $\text{Cu(NO}_3)_2\cdot6\text{H}_2\text{O}$, copper acetate monohydrate, $[\text{Cu(CH}_3\text{COO})_2\cdot\text{H}_2\text{O}]$ and $\text{CuSO}_4\cdot5\text{H}_2\text{O}$ were purchased from M/S. Aldrich Chemical Company, Milwaukee, U.S.A. Mesotetraphenyl porphine copper (II), $\text{C}_{44}\text{H}_{28}\text{N}_4\text{Cu}$ was procured from M/S. Sigma Chemical Company, St. Louis, U.S.A. DEAE-Sephacel and Sephadex G-75 was purchased from M/S. Bio-Rad Pharmacia, Germany.

Before the use of copper (II) acetate monohydrate, for X-ray absorption measurement, it was recrystallized from warm, deionized water to yield turquoise-colored crystals.

Anhydrous copper(II) propionate, $[\text{Cu(CH}_3\text{CH}_2\text{COO})_2]$ was made by diluting about 15 ml of propionic acid to about 40 ml with deionized water and adding about 3 gm of cupric carbonate a little at a time. The solution was heated to about 70°C and stirred overnight, then cooled in ice and the green crystals filtered off.
and dried over P₂O₅.

The complexes, tetrakis(imidazole)copper(II) nitrate, tetrakis(imidazole)copper(II) perchlorate, tetrakis(imidazole)copper(II) chloride and tetrakis(imidazole)copper(II) iodide were synthesized by the literature methods¹⁻⁴. Only a brief account of these methods is given here. Tetrakis(imidazole)copper(II) nitrate, was made by the reaction of an ethanolic solution of Cu(NO₃)₂·6H₂O and imidazole in 1:4 molar ratio. After evaporation for 20 hours purple crystals of Cu(imid)₄(NO₃)₂ (imid = imidazole) were formed.

Tetrakis(imidazole)copper(II) perchlorate, Cu(imid)₄(CIO₄)₂ was prepared by adding the solutions of sodium perchlorate to the solutions of copper(II) salt and imidazole. The reaction mixture was kept in air for four days for slow evaporation to obtain blue-violet crystals of Cu(imid)₄(CIO₄)₂. Tetrakis(imidazole)copper(II) chloride and tetrakis(imidazole)copper(II) iodide were made in a similar way by adding appropriate solutions of sodium and copper salts to imidazole.

Anhydrous copper formate, Cu(HCOO)₂ was synthesized by reaction of about 2 gm of cupric carbonate with 35 ml of 90 % formic acid. The solution was stirred and kept warm overnight, then cooled and the blue crystals formed were filtered and dried over P₂O₅.

Copper(II) citrate dihydrate, Cu₂OC(CH₂COO)₂COO.2H₂O was prepared by the method of Mastropròolo et al⁵ by dissolving 4.2 gm of citric acid, 3.4 gm of cupric chloride and 24 gm of urea in 100
ml of deionized water. The solution obtained in this reaction was filtered and then heated in an oven at about 85°C for about 25 hours. The green, insoluble crystals were filtered and air-dried.

Copper chromite was obtained by heating the mixture of copper oxide and Cr₂O₃ in 1:1 proportion at 950°C.

Ba₄NaCuO₄(CO₃)₂ was prepared by the method of VerNooy and Stacy. A mixture of 0.5 gm of CuO, 0.33 gm of Na₂CO₃ and 1.9 gm of BaCO₃ was added to 14.0 gm of Ba(OH)₂ and 2.5 gm of BaCl₂·2H₂O (BaCl₂·2H₂O was dried at 110°C before use). All the powders of above compounds were mixed thoroughly and the mixture was kept in a crucible with a tightly-fitting lid. The crucible was then placed in the furnace and heated from room temperature to 750°C over 2.5 hours, held at 750°C for 5 hours and cooled gradually to 600°C over 40 hours. The crucible was then removed from the furnace and placed in a dry nitrogen atmosphere. After two days, deep red colour crystals formed a thick layer on the surface of the solidified melt and were isolated mechanically. These crystals are found to be moisture-sensitive and therefore utmost care was taken not to expose them to air. The powder of these crystals was packed in a rectangular plastic cell made for performing X-ray absorption measurements.

Copper (II) tetraamine sulphate monohydrate, Cu(NH₃)₄SO₄·H₂O, was prepared by adding an excess of ammonia to an aqueous copper(II) sulphate solution. The deep purple crystals were precipitated out by adding ethanol to the solution.
In addition to above compounds, a group of four model compounds namely, trimethylimidazole copper dichlorate, KCuF$_3$, CuSeO$_3$.2H$_2$O, tetrakis(pyridine N-Oxide) copper(II) and tetrafluoroborate, [Cu(C$_5$H$_5$NO)$_4$(BF$_4$)$_2$] have also been prepared, the methods of preparation of which are well documented $^7$-$^{12}$.

Cs$_2$CuCl$_4$, Cu(II)diethylidithiocarbamate, Cu(II)isopropyl dithiocarbamate, and KCu(biuret)$_2$ were obtained from the Biochemistry Department.

II.2.2 PREPARATION OF CUPRO-ZINC SUPEROXIDE DISMUTASE

There are different methods available $^{13}$-$^{17}$ to isolate superoxide dismutase (SOD) from bovine blood. The following method which is similar to those of Gartner et al. $^{15}$ was used to extract SOD since it gives high yield and is less time consuming.

Bovine red blood cells were separated from plasma and washed with 0.9% NaCl. The cells were lysed after dilution with 1-2 vol. of tap water. The lysate was dialysed for 14 hours and heated at 70-80°C for 10-15 minutes at pH 7 while stirring was maintained. Roughly 90% of the haemoglobin is removed by this procedure, were as 60-70% the SOD was recovered. Separation of the soluble fraction from the precipitate was performed by centrifugation. Before chromatography on DEAE-Sephacel the pH was adjusted to 6.5 with acetic acid. Under these conditions the residual haemoglobin passed almost completely through the ion-exchanger. The bound nonhaemoglobin proteins were eluted with a linear NaCl gradient.
(0-200 mM). The superoxide dismutase fraction was concentrated by freeze-drying or membrane filtration. Further separation of contaminant proteins as well as desalting was accomplished in one step after passage through a Sephadex G-75 column equilibrated with distilled water. The homogeneous enzyme was freeze-dried. A final yield of 50% of the originally present enzyme is achieved in this process.

Anion derivatives superoxide dismutase were prepared as follows. To a 10 mmol dm\(^{-3}\) solution of native enzyme prepared above was added an amount of chelex-treated sodium azide or potassium cyanide so as to result in a final concentration of anionic ligand of 100 and 60 mmol dm\(^{-3}\) of azide and cyanide respectively.

The activity of SOD in bovine blood was checked by the method of Carpo and Tierney\(^{20}\). The measurement of activity or the assay procedure involves the inhibition of epinephrine autoxidation in an alkaline medium as described by Mishra and Fridovich\(^{21}\). 6 ml of bovine blood was added to 0.9 ml of sodium citrate and refrigerated centrifused for 15 min at 5000 - 6000 x g (i.e., 4500 - 5000 rpm). After removing the plasma and buffy coat, red blood cell pallate was washed with phosphate buffer saline. Red blood cells were hemolysed by adding 10 volumes of phosphate buffer containing 0.1 mM EDTA at pH 7.8. RBC membrane was then removed by centrifugation at 20,000 x g (i.e. 18000 rpm). Hemolysate was collected and precipitation of hemoglobin was done by adding chilled 3:1 ethanol-chloroform mixture. After incubation of 30 min at 4 °C,
hemolysate was subjected to centrifugation at 3000 rpm for 20 min. Supernatant was collected and kept frozen till assay was done. When kept frozen the enzyme remains stable for at least two weeks. The final assay mixture for the autoxidation contained in a volume of 3 ml epinephrine (3 x 10^{-4} M), EDTA (1 X 10^{-4} M) and 0.05M sodium carbonate at pH 10.2.

The reaction was started by adding the epinephrine solution to the assay mixture and the change in the extinction coefficient was followed at 480 nm in a Baush & Lamb Spectrophotometer. The rate of change of extinction coefficient as reported by Mishra and Fridovich\textsuperscript{21} was 0.025/min at 25 °C. In our experimental conditions we consistently found an absorbency change of 0.024/min. SOD activity was determined by measuring the inhibition of epinephrine autoxidation after addition of the supernatant or purified enzyme. The readings were taken at 30 seconds interval and enzyme assay was always done in duplicate. In the assay, where supernatant was added to the reaction mixture, a latent period of a few minutes existed, before extinction coefficient begins to increase linearly and this was taken as the initial rate of the enzyme reaction. The same phenomenon was noted by Carpo and Tierney\textsuperscript{20} in their experiments. The enzyme activity was expressed in arbitrary units causing 50% inhibition in the reaction mixture of 3 ml. under our experimental conditions. Purity of SOD was checked by SDS polyacrylamide gel electrophoresis with standard SOD purchased from M/s Sigma Chemical Company, U.S.A.
II.2.3 PREPARATION OF COPPER GLUCURONATES AND COPPER COMPLEX FORMED FROM *PSEUDOMONAS AERUGINOSA*

The following complexes were prepared using a method similar to that of Traube and Kuhbier\textsuperscript{18}: magnesium-copper glucuronate, barium-copper glucuronate and copper galacturonate. To 2 gm of glucuronic acid dissolved in ion-free water, 1.8 gm of freshly precipitated cupric hydroxide was added and the mixture treated with 50 ml of 9% NaOH until a clear, deep-blue solution was obtained. To prepare the barium salt, 25 ml of 10% BaCl\textsubscript{2} was added to the deep-blue solution when the light blue barium copper-glucuronate precipitated. The barium copper-glucuronate (sparingly soluble in water) was washed with water, ethanol and finally acetone until free from residual NaOH. It was dried in a desiccator over H\textsubscript{2}SO\textsubscript{4} and finally stored in a vacuum desiccator. The magnesium copper-glucuronate is soluble in water and was, therefore, washed with 2 volumes of 25 ml of ethanol, followed by acetone and ether; this was also stored under vacuum over H\textsubscript{2}SO\textsubscript{4}.

Barium copper-galacturonate was prepared from galacturonic acid in a manner similar to the preparation of barium copper-glucuronate. A mucoid *Pseudomonas aeruginosa* strain 9501 which was used in all the experiments was donated to us by Professor R. Magee, Department of Inorganic and Analytical Chemistry, La Trobe University Bundoora, Victoria Australia. A chemically-defined minimal salts medium was used to cultivate the bacteria\textsuperscript{19}. Copper sulphate was used in all the uptake experiments.
As mentioned earlier, cultures were grown in the minimal salts medium. After 23 hours incubation with Cu\(^{2+}\), the extracellular material was removed from the bacteria either by treatment for 30-60 sec in a blender or by shaking for 30 minutes at 310 K with 10% (V/V) ethylene glycol. The cells were removed by centrifuging at 2000 g for 2 hours at 293 K. The slime was precipitated from the supernatant by addition of an equal volume of ethanol. After 24 hours at 277 K, the supernatant was decanted and the slime redissolved in the minimum volume of hot (333 K) 0.1 M KOH. After cooling (290 K), the slime was reprecipitated with an equal volume of ethanol. The precipitate was kept overnight at 277 K, the supernatant decanted and the slime washed (90% V/V ethanol) followed by ether. After drying over P\(_2\)O\(_5\) and KOH in vacuum, the copper-containing slime was stored in a desiccator over P\(_2\)O\(_5\). The copper complex present in the slime was extracted by dissolving the slime in 2N saline solution. After filtration, the insoluble copper complex was washed with two volumes of 25 ml of ethanol, acetone and finally ether. The complex was then stored under vacuum over H\(_2\)SO\(_4\).

11.2.4 PREPARATION OF MODEL VANADIUM COMPOUNDS AND COMPLEXES.

Vanadium metal foil, VC, VN, VO, V\(_2\)O\(_3\), V\(_2\)O\(_4\), V\(_2\)O\(_5\), Na\(_3\)VO\(_4\), V\(_2\)S\(_3\), Vanadium tetraphenylporphine, Vanadium phthalocyanine, vanadyl acetylacetonate were obtained commercially. Other model vanadium compounds investigated in this work were synthesized by
Anjli Chhikara et al.** for X-ray spectroscopic study of complex oxides and organometallic compounds.

Vanadium complex of the tridentate schiff base ligand N-salicylidene-N-(2-Hydroxyethyl) ethylenediamine (designated as HSHED) studied in this work was synthesized by the method of Li et al.** Salicylaldehyde (2 ml, 18.8 mmol) and N-(2-Hydroxyethyl) ethylenediamine (1.9 ml, 18.8 mmol) were added to 75 ml of degassed methanol and allowed to react for 1 hour. Vanadyl sulphate hydrate (3.74 gm, 18.8 mmol), dissolved in 50 ml of water, was added to this solution, and after about 3 hours the reaction solution turned blue with a small amount of blue solid precipitating. At this point, NaOH (1.5 g, 37.6 mmol) was added to the mixture, and then the solution was kept in air, and the reaction was continued for 14 hours. At the end of this process, a Yellow colour compound [VO₂(HSHED)]₂ was precipitated.

II.2.5 PREPARATION OF (Me₄N)[VFe₃Cl₃(DMF)₃].2DMF

Following the method of Kovacs and Holm, a VFe₃S₄ cluster, (Me₄N)[VFe₃Cl₃(DMF)₃].2DMF was synthesized in the laboratory (DMF = dimethyl formamide). A slurry of (NH₄)₃[VS₄] (12 mmol) and equimolar Me₄NBr in 300 ml of DMF was allowed to react with 52 mmol of anhydrous FeCl₂ under nitrogen atmosphere, giving an intense red solution which slowly turned brown. After 16 hours workup of the reaction mixture obtained above, a black crystalline solid,
(Me₄N)[VFe₃Cl₃(DMF)₃]·2DMF was obtained.

II.2.6 PREPARATION OF VANADIUM NITROGENASE

The experiments were carried out with the Azotobacter Chroococcum strain was obtained from AFRC Unit, University of Sussex, UK. Organisms were grown in air at 30°C and nitrogenase activity was measured in cultures by following H₂ evolution as described by Robson et al.²⁵ As mentioned above, the organisms were grown in the medium in presence of 40 µM VOSO₄. Cells were collected during the long phase, suspended in 50 mM HEPES buffer at pH 8.00 and disrupted by French pressure cell treatment. Nitrogenase was purified under strictly anoxic conditions and separated into two components by using DEAE-Sephacel ion exchange chromatography in 50 mM Tris-HCl buffer pH 8.0. Both proteins are then subjected to a linear NaCl gradient (0.25-0.4 M NaCl). Act fraction was concentrated by freeze drying method and used for our X-ray spectroscopic work.

II.3 X - RAY ABSORPTION MEASUREMENTS

We describe below the apparatus used in this investigation to record the X-ray absorption spectra of a copper and vanadium their pure metallic forms, several model compounds and in a few biological complexes. It is to be noted here that some of the spectra were recorded using very high intensity X-ray source. This is particularly important where the concentration of the absorbing
metal ion is very low.

II.3.1 X - RAY SPECTROMETER

Our basic X-ray spectrometer is a horizontal diffractometer with attached tube stand. The major modification is an improved crystal (monochromator) support for optimum alignment of the diffracting crystal (Fig.II.1). This spectrometer is used with a conventional X-ray diffraction generator and tubes (W, or Cu target) chosen to provide a high intensity continuum in the region of absorption edge to be measured, while missing the intense characteristic lines. The spectrometer is used with a goniometer radius of 18.75 cm using simple set-back brackets and an X-ray tube takeoff angle of approximately 3° (adjusted for maximum diffracted intensity). The first slit is used to limit the angular divergence of the X-ray beam, and the Bragg-Brentano parafocussing conditions and slit position require the focussing slit (exit) slit to be approximately the same size. Between the two slits, a single-crystal monochromator Bragg diffracts a narrow band of the incident X-rays and adds its diffraction pattern to the divergence of the beam. The vertical divergence is limited to ± 2° by a Soller slit located before the exit slit. Thus the resolution function depends primarily on the size of the slits and crystal diffraction pattern. Our usual method of operation employs 0.05-mm slits (0.025° divergence) in the 2θ range from 15° to 45° and 0.1mm (0.5° divergence) slits for angles greater then 45° with overlap as
Fig. II. 1 Schematic diagram of X-ray Absorption Spectrometer
experimentally required. Hence resolution power defined as
\((\tan \theta) / \Delta \theta\) (or \(\lambda / \Delta \lambda\)) is approximately 1000 - 2000. The diffracted
intensity from the continuum operating at full recommended tube
power is typically \((1-10) \times 10^3\) photons per second before passing
through the sample. For the case of 0.05 mm slits at \(2\theta = 45^\circ\) and a
LiF crystal \((2d = 4.026\text{Å})\), the energy band width, assuming a
rectangular response function, received at the exit slit would be \(<
4\text{eV} (\Delta E = E \cot \theta \, \Delta \theta)\), however, the intensity distribution of the
radiation falling on the exit slit has the usual diffraction
profile and the Rayleigh resolution criterion suggests that the
spectrometer would be advanced in angular increments of 1/2 the
angular width of the exit slit, i.e. \(\Delta 2\theta = 0.01^\circ\) for 0.05 mm slits.

The mode of operation is as follows: For each spectrometer
position, \(I\) and \(I_0\) are measured (preset count mode) and stored on a
floppy disk, the spectrometer is advanced to the next \(2\theta\) position,
and the sequence is repeated. A separate scalar is used to generate
a running number for each subsequent pair \((I, I_0)\). Knowledge of the
start position and the \(2\theta\) stepping increment allows calculation of
X-ray wavelength for any data pair. The spectrometer stepping motor
and absorber changer are activated and synchronized by the X-ray
scalar print-out command. It was found that the mechanical accuracy
of the absorber placement mechanism limited the precision to \(\sim 0.3\%\)
, thus \(10^5\) photons were recorded for each \(I, I_0\) and increased
precision to 0.1% was achieved by averaging multiple passes. The
problem of coincidence loss in the X-ray detecting electronics was
corrected using the method of Short$^{27}$ and Burbank$^{28}$. If uncorrected, intense emission lines from X-ray tube leave an image in the data, which can be mistaken for EXAFS.

The flanged tube stand attached to the spectrometer allowed the X-ray tube to be translated, rotated and inclined with respect to the spectrometer circle. The alignment procedure consisted of locating (by means of these adjustments) the most intense spot on the X-ray tube target so that it was directed through narrow aligned entrance and exit slits at $\theta = 0^0$ as measured by a protected X-ray detector. The crystal monochromator was then inserted and a suitable characteristic line chosen for final crystal adjustment. At the calculated $2\theta$ the translation, tilt and rotation ($\theta$) adjustments of the crystal holder were used to obtain peak diffracted intensity of standard line which located the diffracting volume of the crystal at the centre of the spectrometer. Further refinement of the alignment was not necessary if the procedure described in the section on precision and accuracy of the energy scale was followed.

11.3.2 MONOCHROMATOR CRYSTALS

The usual discussion of monochromators for X-ray spectrometers emphasizes high resolution with narrow crystal rocking curves and multicrystal spectrometers. Our requirement stresses high X-ray intensity at moderate resolution for good statistical accuracy of measured EXAFS. Although better resolution would probably show more details particularly near the edge, there is an inherent width
(~20eV) in the EXAFS due in part to temperature smearing as well as lifetime broadening \(^{29}\) e.g., 1.5 eV for the Cu K edge.

Given the flat crystal geometry, this experiment is intensity limited by the inherent luminosity of X-ray tube and the diffraction efficiency of the monochromator. A given monochromator has a diffractive dispersion called its "rocking curve" which is the angular width of the diffracted beam. When the crystal is exposed to a continuum of diverging radiation, it selects from the total flux just that angular range of wavelength \(\Delta \lambda\), which is its rocking curve width, and diffracts a narrow band toward the exit slit, thus a crystal with very a narrow rocking curve will diffract comparatively few photons. A crystal with a wider rocking curve than the divergence of the slit system will smear the diffracted beam over the exit slits. The optimum condition is obtained when the divergence of the slit system and rocking curve of monochromator are approximately equal.

The efficiency of diffraction and the rocking curve width of diffraction crystals may be modified by appropriate treatment. LiF(200) and Si(220) are particularly workable crystals in this respect. The integrated reflection coefficient for nonpolarised radiation, which is the area under the crystal rocking curve, has been calculated as a function of wavelength for the two extremes of the crystal perfection, a perfect crystal and an ideal mosaic crystal \(^{30-32}\). The measured values of selected and treated crystals are also shown \(^{31-32}\). Note that the treatment increased the photons
diffracted by a factor of 3. The time necessary to obtain a precision measurement was reduced proportionally. The treatment consisted of vigorous sandling on rough paper to drive dislocations into the crystal followed by successively finer paper to 600 grit to provide a smooth surface. Part of the damaged surface layer was then removed by an etching procedure. After one minute in concentrated HF, a chemical polish consisting of 2-vol.% NH₄OH in H₂O at 26°C with vigorous agitation was used to remove the surface at about 1µm/min. The crystal was checked repeatedly until rocking curve width narrowed to that of the desired slit size. We have used similar techniques with Si and quartz crystals with some success.

II.3.3 PREPARATION OF ABSORBERS

The absorbers were prepared in a variety of ways, malleable metals were rolled (2-5 µm); some materials were evaporated onto mylar or thin Al foil, soluble materials were dissolved and then absorbed and dried on a filter paper, many materials were ground to pass 400 mesh, mixed with a vacuum grease and then cast on a smooth substrate. When dry, the casts were sandwiched between thin transparent adhesive tapes for support and attached to the sample holder. For liquid samples, a rectangular sample holder having mylar windows was made. The optimum absorber thickness considering contrast, measurement time, and primarily the coincidence counting error problem, was attained when I/I₀ ~ 1/3 on the high absorption side of the edge. For experiments in which the element of interest
was very dilute, thickness was used such that $I/I_0 \sim 1/10$. For example, with this thickness a satisfactory $K$ absorption pattern of copper-zinc superoxide dismutase was obtained in 15 passes. For liquid samples, a rectangular sample holder having mylar windows was made.

II.3.4 PRECISION AND ACCURACY OF THE ENERGY SCALE

The kinetic energy of the ejected photoelectrons $E$ must be established accurately in order to evaluate the natural EXAFS variable $k$. For every experiment, characteristic and/or impurity lines from the X-ray tube occurred and were used as standard reference points to calculate an effective lattice constant for the monochromator to establish the energy scale at the accuracy to which they were known. Many elements were present as an impurities on the X-ray tube anode; W, Cu, Ni, Fe, and Mn are usually present, plus the characteristic line from the primary anode element in multiple orders of diffraction. Replicate experiments established a precision of $\sigma = 75$ ppm ($\pm 0.7$ eV at the Cu K edge) as compared to 40 ppm typical calibration lines. By using the calibration lines to calculate the lattice constant for every set of data, the requirement was removed for corrections involving the diffraction process in the monochromator crystal such as a temperature correction, Lorentz-polarization correction, refraction and various errors due to misalignment. The value of $E$ associated with each data pair was calculated from
\[ E = 12398.52/2dsin\theta \text{ ev} \quad (II.1) \]

The data collected from the EXAFS apparatus consisted of the time to collect a preset number of counts with the absorber in (T) and out (T_0) of the spectrometer.

The preliminary data processing program mentioned in Chapter III tabulated the initial data and calculated the X-ray energy, the kinetic energy of photoelectron, and the total absorption. All the other details are given in Chapter III.

II.4 OTHER MEASUREMENTS

In addition to X-ray absorption spectroscopic technique, we have also employed four more techniques, X-ray Photoelectron Spectroscopy (XPS), Infrared (IR) spectroscopy, Electron Spin Resonance (ESR) spectroscopy and X-ray diffraction (XRD) for the characterization of a few important copper compounds. A brief description is given in the following few paragraphs about the measurements carried out using these techniques.

Infrared spectra were recorded on a PYE UNICAM SP 1100 double beam infrared spectrometer. The spectra of the samples were taken as mulls in Nujol and Fluorolube in the range 4000 cm\(^{-1}\) - 400 cm\(^{-1}\).

X-ray photoelectron spectra of powdered copper samples were recorded using an ESCA 3 Mark-II spectrometer of VG Scientific Limited, UK, fitted with a sample preparation chamber. The
operating vacuum in the chamber was $10^{-10}$ torr. This chamber was fitted with an argon ion gun and a quadrupole mass spectrometer. The radiation employed was Al K$_\alpha$ (1486.6 eV). The spectrometer was calibrated with reference to the binding energy of Au (4f$_{7/2}$) at 83.7 eV.

ESR measurements were made on a Varian E-13 spectrometer with a variable temperature arrangement and capability for modulation at 100 kHz, alternatively and simultaneously, at lower frequencies. Magnetic field positions were determined using diphenylpicryl hydrazide as the internal standard. The instrument settings for copper samples investigated in this work were: microwave frequency 9.117 GHz, microwave power 50 mW, modulation amplitude 0.63 G, time constant 1 s and temperature -196 °C and scanning time for each sample 8 min. The uncertainty in the g values is estimated to be ± 0.004.

All the samples prepared in the laboratory were investigated by powder X-ray diffraction technique using an automated Rigaku DMAX II-C X-ray diffractometer with Cu K$_\alpha$ radiation generated at 40 kV and 20 mA. X-ray diffraction patterns were collected at scan speeds of 1 degree per minute. The observed relative intensities of Bragg peaks and the corresponding d-values of our compounds agree very well with those reported in JCPDS data files. It may be noted here that before carrying out X-ray diffraction analysis, the compositions of the compounds synthesized were determined by chemical analysis using standard methods.
REFERENCES

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<td>Si(311) 3.276</td>
<td>43.839°</td>
<td>87.679°</td>
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<td></td>
<td>(43°50'23&quot;)</td>
<td>(87°40'46&quot;)</td>
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<tr>
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<td>Si(400) 2.715</td>
<td>56.695°</td>
<td>113.391°</td>
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<td></td>
<td>(56°41'43&quot;)</td>
<td>(113°23'27&quot;)</td>
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