

**SYNTHESIS AND CHARACTERIZATION OF INSULIN MIMETIC VANADYL
COMPLEXES AND THEIR BINDING STUDIES WITH BOVINE SERUM ALBUMIN**

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BS-MS Dual Degree Programme

Thesis Supervisor: **Prof.M.Devadas**

By

V.LOUWKHYAA

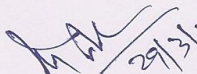
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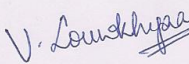
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CERTIFICATE

This is to certify that this dissertation entitled "*Synthesis and Characterization of insulin mimetic vanadium complexes and their binding studies with BSA*" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents original research carried out by Vemulapalli Louwkhya at Department of Chemistry, University College of Science, Osmania University under the supervision of Prof.M.Devadas, Department of Chemistry, University College of Science, Osmania University during the academic year 2013-2014.


Supervisor: Prof.M.Devadas
Professor
Department of Chemistry
Osmania University
HYDERABAD - 500 007.


V.Louwkhya
(20091068)

DECLARATION

I hereby declare that the matter embodied in the report **entitled “*Synthesis and Characterization of insulin mimetic vanadium complexes and their binding studies with BSA*”** are the results of the investigations carried out by me at the Department of Chemistry, University College of Science, Osmania University, under the supervision of Prof M.Devadas and the same has not been submitted elsewhere for any other degree.

V.Louwkhya
(20091068)

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I. ABSTRACT

For over a century, vanadium salts (vanadyl sulphate, sodium metavanadate, ammonium orthovanadate) have been shown to have insulin-like effects in improving the symptoms of Type II Diabetes Mellitus. The discovery by Lyonnet and Martin in 1899 which reported diabetic patients when treated with vanadate (VO_4^{3-}) excreted less glucose in their urine was the first paper which gave an idea that vanadium salts can be used as metallotherrapeutic agents. Later in 1985, Heyliger et al. reported the in vivo insulin mimetic activity of oral vanadate. Further studies confirmed that vanadium compounds are effective for lowering plasma glucose levels in diabetic subjects. Recently, it has been proved that the complexes of vanadium with organic ligands seemed to be less toxic than inorganic vanadium salts. A comparative study between VOSO_4 and $\text{VO}(\text{AA})_2$ (Vanadyl (IV) bis(acetylacetonate)) has shown that organic vanadyl complex performed much better than inorganic salt because of its greater hydrolytic and redox stability. It was concluded that $\text{VO}(\text{AA})_2$ was a more potent stimulator in lipid metabolism compared to VOSO_4 . In the current study, vanadium complexes of substituted β -diketones were selected based on the results of vanadium (IV) bis (acetylacetonate). Different methods were attempted to develop binary (1:2) vanadium complexes. All the complexes are characterized by UV-Vis, IR, Mass spectral studies, conductivity measurements. Potentiometric studies were conducted to examine the stability of complexes at different pH values. Further, the interactions of vanadium complexes with Bovine serum albumin (BSA) were done by dialysis technique. Consequently, binding (absorption) properties were studied using UV-Vis spectroscopy.

Keywords: Vanadyl (IV), β -diketones, BSA.

II. INTRODUCTION

*I became captivated by the edifices chemists had raised through experiment and imagination
–but still I had a lurking question .Would it not be better if one could really ‘see’ whether
molecules as complicated as the sterols or strychnine were just as experiment suggested?
– Dorothy Crowfoot Hodgkin.*

A. Diabetes Mellitus (DM):

It is a chronic disease characterized by high levels of blood sugar resulting from defects in insulin production, insulin resistance or both. This disease is associated with major health risks like kidney failure, blindness, and nerve damage, cardiovascular disease and stroke. The occurrence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and expected to rise to 4.4% in 2030[1]. The major parts of this numerical increase will occur in developing countries. The greatest absolute increase in the number of people with diabetes will be in India. The first ten countries estimated to have the highest number of people living in the diabetes in 2000 and 2030 are listed in table.

Table 1: List of Top Ten Countries in the DM Population

Rank	Year 2000		Projected figure for 2030	
	Country	DM Population (Millions)	Country	DM Population (Millions)
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	US	17.7	US	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Japan	8.9
6	Pakistan	5.2	Pakistan	13.9
7	Brazil	4.6	Brazil	11.3
8	Bangladesh	3.2	Bangladesh	11.1
9	Italy	4.3	Philippines	7.8
10	Russia	4.6	Egypt	6.7

DM is classified into two groups:

Type I DM: Insulin dependent diabetes mellitus (IDDM) which is caused by destruction of pancreatic β -cells.

Treatment: Daily injection of Insulin.

Type II DM: Non-insulin dependent diabetes mellitus (NIDDM) which is caused by ageing, obesity, mental stress or other environmental factors.

Treatment: synthetic oral drugs such as metformin, thiazolidinediones, sulfonyl ureas.

This disease is associated with major health risks like kidney failure, blindness, and nerve damage, cardiovascular disease and stroke. There are several difficulties concerning in preparation of insulin and synthetic drugs. In addition to this, there are many side effects related to them. So, the disease insists to discover new therapeutic approaches. For this purpose, metal complexes have been explored.

Some metal ions in treating DM:

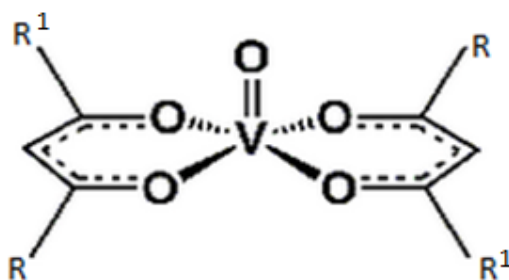
Several metal ions including Vanadium(V), Chromium(Cr)[2], Manganese(Mn)[3], Cobalt (Co)[4,5], Zinc (Zn)[6], Selenium(Se)[7,8], Molybdenum (Mo)[9], and Tungsten (W)[7,10] and their complexes have been reported as showing insulin-mimetic activity in-vitro experiments and to exert positive treatment effects on experimental animals and subjects with DM. Vanadium was chosen for the current work.

B. Vanadium chemistry:

i. Coordination chemistry:

Most coordination numbers of oxo vanadium (IV) complexes are 5 & 6 based on the VO^{+2} moiety [11]. Independent of oxidation state, the geometry for five-coordinated compounds is commonly square pyramidal, usually slightly distorted. The trigonal bipyramidal arrangement is less frequent. Typical bi to tetra dentate ligands used for the stabilization of oxo-dioxovanadium center contains O-donor (phenolate &

carboxylate) and N-donors (aromatic/aliphatic amines & imines). The chemistry of the square pyramidal geometry has been well established with oxygen of the oxo vanadium (IV) unit in the axial position and the vanadium atom lying above the plane defined by the donor atom of the four equatorial ligands. These square pyramidal complexes generally exhibit a strong tendency to remain five coordinate [12]. The absorption band due to V=O stretching vibration of oxo vanadium (IV) is usually absorbed at higher wave number compared to those of vanadate complexes. The V=O stretching vibration, however, is susceptible to a number of influencing factors including electron donation from basal plane ligand atoms, solid state effects and coordination of additional solvent molecule.



R and R¹ can be CH₃, CF₃, C₆H₅, t-butyl, thienyl

Fig 1: Proposed structure of vanadium (IV) β -diketones.

ii. *Toxicity:*

Vanadium toxicity depends on specific chemical form, oxidation states, administrative route, period and doses, as well as types of organisms studied

The chronic toxic strength patterns of Vanadium compounds follow the below order:

Inorganic salts > Organic vanadium compounds

(Soluble salts > Insoluble salts) > V (V) Organic compounds > V (IV) Organic compounds.

In the case of Vanadium organic Compounds, the toxicity also depends on the donor atom coordinated to vanadium. NN, OO or NO are less toxic than compounds with donor sets of NS, OS or SS, irrespective of the vanadium oxidation state. The toxicity tends to decrease as the valence decreases.

C. Vanadium and diabetes:

In 1899, the very first report showed that vanadium in +5 had effective glucose lowering properties. The French Physicians administered sodium metal vanadate (NaVO_3) to improve the health of patients suffering with Diabetes Mellitus (DM) [13]. A series of studies in 1979 & 1980 demonstrated the ability of Na_3VO_4 (sodium orthovanadate), NaVO_3 (sodium metavanadate) and VOSO_4 (vanadyl sulphate) to have insulin like biological effects of glucose uptake & metabolism in skeletal muscle and adipose tissue *in vitro* [14-16]. The subsequent discovery of *insulin* [17] in 1921 directed the focus of research away from inorganic compounds. Further studies on vanadium complexes dramatically accelerated when Heyligher et.al [18] in 1985 first reported the successful treatment of streptozotocin injected insulin-deficient diabetic rats with oral vanadate. Blood glucose concentrations were lowered without a change in insulin concentration substantiating the ability of vanadate to mimic insulin *in vivo*. Since then extensive studies exploring vanadium chemistry including the synthesis of novel compounds as well as the biological effects of vanadium on cells and tissues *in vitro* and *in vivo* have been performed.

Vanadium as vanadyl sulphate (VOSO_4) is believed to regulate fasting blood sugar levels and improve receptor sensitivity to insulin. The vanadyl sulphate appears to be a useful intervention for type II diabetic individual with insulin resistance. The vanadyl

sulphate has been reported to be 6-10 times less toxic than vanadate [19]. The very interesting study was conducted recently to know the insulin enhancing effect of vanadium in its different oxidation states viz 3+, 4+ and 5+. The chosen ligand was dipicolinic acid and 4-hydroxydipicolinic acid. The metal salts used were VCl_3 , $NaVO_3$ and $VOSO_4$. The authors have concluded that the effectiveness of metal complex depends on oxidation state of vanadium and nature of ligand e.g. vanadium in +4 state with maltol, and vanadium in +5 state with dipicolinic acid was effective [20]. Shamoon[21] has shown that vanadylsulphate ($VOSO_4$) does not enhance insulin action in patients with type I diabetes, but selectively improves insulin action in subjects with insulin resistance type II diabetes. Fawcett.JP[22] stated that oral vanadyl sulphate does not effect blood cells, viscosity or biochemistry in human beings. Fantus[23] has demonstrated that vanadate mimics insulin directly, enhances sensitivity to insulin and prolongs insulin biological effects, then promoting insulin acting by at least three physiological mechanics. These observations support the notion that vanadate or related vanadium compounds may be particularly effective therapeutic agents for insulin resistant forms of DM (Type II) as suggested by Shamoon.

The mechanism of insulin action is a challenging problem. From 1921, the year of discovery of insulin, scientists have been focused their research on the metabolic changes that occur during insulin stimulation- first at the whole animal level and later at the cellular level[24,25]. These studies suggested that the process. (viz, glucose uptake, lipid synthesis, protein synthesis and glycogen deposition) are controlled by insulin, but the principle cause of insulin resistance has not yet been identified. Recently, reduced insulin receptor tyrosine kinase activity has been reported in cells for type II diabetes. As far as the mode of action of vanadyl compounds are concerned, it has been proposed that the vanadium inhibit protein tyrosine phosphatases (PTP-ases)[26,27]. A tentative role of insulin and vanadium in transporting glucose is given in **Fig. I**. The cell membrane contain trans-membrane receptors for insulin, which are protein tyrosine kinase (PTK) .When insulin docks the receptor outside, tyrosine moiety of receptor projecting inside the cell is phosphorylated, giving rise to a signal transduction cascade (represented by arrow in fig 1) which leads to glucose intake by a glucose transporter symbolized by an “opening door”. In absence of insulin or in the case of insulin resistance (**Fig I.d-f**),

another protein called protein tyrosine phosphatase (PTP-ases) catalyses the hydrolytic rupture of phospho–ester bond, thus diminishing the signal transduction, symbolized by “closing door” for intake of glucose. This hydrolysis is fully effective in the absence of insulin. The extent of tyrosine phosphorylation inside cell reflects the perfect tune of balance between action of two proteins (PTK and PTP-ases) i.e. phosphorylation and dephosphorylation (essence of the mechanism).

Vanadium's close resemblance with phosphate allows it to enter the cell via phosphate & sulphate channels. It has been proposed evidenced that vanadate inhibits the action of PTP-ases as a result phosphoester bond is not broken thus signal transduction remain intact allowing glucose to enter the cell (**Fig I.d**). Alternative mechanism also proposed in which metal itself may esterificate effectively with tyrosinase of the insulin receptor (**Fig. I.f**)[28]. A non-membrane protein-tyrosine kinase (PTK) may be activated by vanadium (**Fig.I.e**).

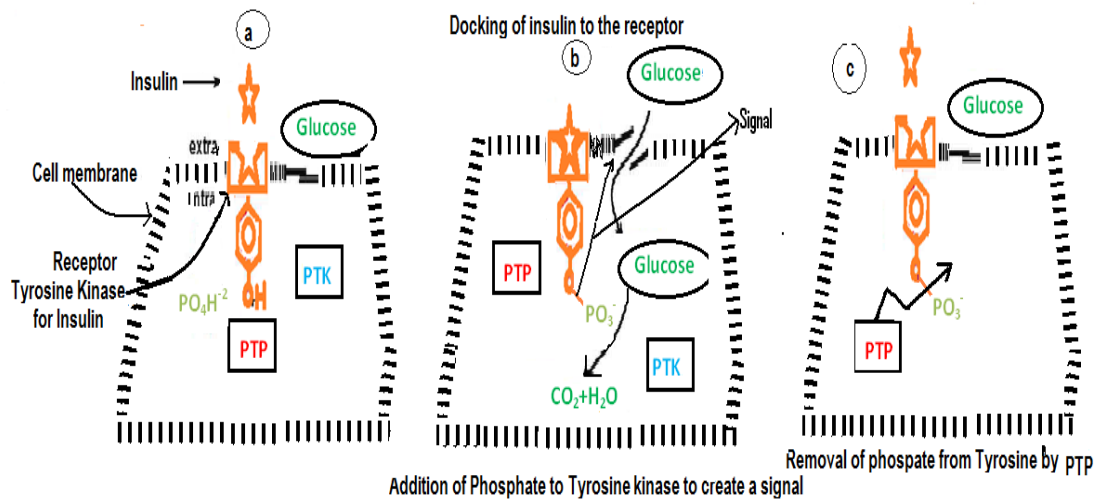
Protein tyrosine kinase (PTK): It is an enzyme which transfers a phosphate group from ATP to protein in a cell. It functions as 'on' or 'off' switch in many cellular functions.

Protein tyrosine phosphatase (PTP): It is an enzyme which removes the phosphate group from amino acid tyrosine. Tyrosine Kinase has an associated protein tyrosine phosphatase for removal of phosphate group from tyrosine.

Phosphorylation: Kinase is a type of enzyme that transfers phosphate groups from ATP to specific target molecules. This process is called phosphorylation.

Dephosphorylation: Removal of phosphate groups from target molecules by an enzyme called “phosphatase”. This process is called dephosphorylation.

Mechanism of Insulin in glucose metabolism/regulation



Vanadium compounds and their possible mechanism in glucose metabolism/regulation

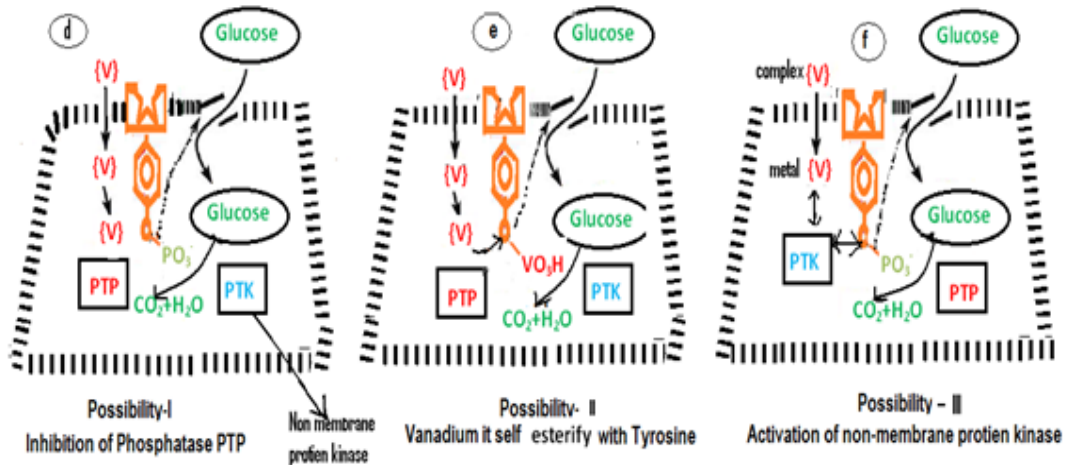


Fig 2: Insulin and Vanadium complexes in their possible proposed mechanism in glucose metabolism/regulation.

D. Scope and objective of the present work:

The vanadium metal complex with organic ligands instead of pure different vanadyl salts offers more versatile means of delivering the metal. In other words, vanadium in the form of organic metal complexes appears in many ways to be superior to the inorganic vanadate or vanadyl in terms of the absorption, distribution, tissue uptake, retention and insulin mimetic activity. Thus the properties of the metal complex may be improved by the correct choice of ligand and useful therapeutic effect may be achieved at lower doses. Bisacetylacetonatoxovanadium (IV) has already been reported for its insulin mimetic activity [29-32]. M.J. Brady et al. reported that $\text{VO}(\text{AA})_2$ potentiates tyrosine phosphorylation of the insulin receptor [33]. This complex was referred as standard (parent compound). This ligand formed stable neutral metal complexes with following optimum combination of

- (a) Water solubility
- (b) Reasonable hydrolytic stability and
- (c) Significant lipophilicity.

In order to be effective drug, the vanadium compound should fulfill the following preconditions:

- Hydrophilicity
- *Lipophilicity* should be balanced by an appropriate design of ligand system in order to allow absorption and transport in the blood stream.
- Complex should be *stable*; atleast to the extent where it partially survives in the acidic conditions inside the stomach.
- The ligand sphere should contain a site for bio-recognition in order to facilitate the trans-membrane transport.
- The complex should contain an empty or easily accessible (by ligand exchange) site for coordination to the target molecule.
- Last but not the least, it should have minimized toxicity.

Research work continued towards the less toxic form of vanadium with lower oxidation state in order to control and enhance its transportation via biological membranes in the body and to reduce the required effective dosage below the toxic

level, numerous complexes with different structures were studied. There are multitudinous papers in chemistry and biology regarding this topic till date. Very recent papers demonstrated the role of vanadium in diabetes and its cardiac complications, renal function and cataract etc.[34-36]. The nature has still kept all the global scientists busy in solving this puzzle. While currently available, vanadium compounds remain as experimental probes to examine the mechanism of altered insulin action; more studies are needed to establish any role for their clinical usage. Hence it has been proposed to prepare and characterize vanadium metal complexes with different coordination environments around vanadyl ion. We chose substituted β -diketones as ligands for the present study. Comparative studies were done with the parent compound, vanadyl bis(acetylacetonate) (**VO(AA)₂**). Main concern of the work is to observe the role of substituted groups attached to the acetylacetone backbone. Synthesis and characterization was done and preliminary study of the interactions of vanadium complexes with bovine serum albumin, most abundant plasma protein was investigated with the aid of UV-vis spectroscopy. BSA acts as a carrier for fatty acids, nutrients, metabolic products. It also binds and neutralizes endogenous or exogenous toxins by means of hydrogen bonding, hydrophobic, electrostatic and metal interactions[37]. The mechanism by which they interact was not known clearly. Since vanadium complexes take part in glucose uptake and metabolism, binding properties of vanadium complexes with BSA was inspected. Binding studies were done with dialysis method, which can be used to introduce or remove small molecules from the sample because they move across the semi-permeable membrane in both directions. Hence it is useful, inexpensive technique for drug binding studies.

III. Experimental

Materials and Methods

A. Reagents

i. Reagents used for synthesis:

$\text{VO}_2\text{SO}_4 \cdot x \text{H}_2\text{O}$ (vanadyl sulphate), Diketones from Sigma Aldrich, Sodium carbonate, sodium acetate, sodium chloride from Merck were purchased. All the organic solvents used were from Sigma Aldrich. All the chemicals and the solvents were of analytical grade and used without further purification.

ii. Reagents used in potentiometry:

$\text{VO}_2\text{SO}_4 \cdot x \text{H}_2\text{O}$ (vanadyl sulphate), β -diketones, KNO_3 , NaOH .

iii. Reagents used in dialysis:

Prepared complexes, BSA from Sigma Aldrich, PBS buffer.

B. Apparatus and Instruments:

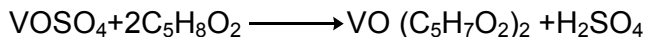
UV-vis spectra were done on Shimadzu UV-3600 series. IR spectra was recorded using Shimadzu FTIR-8300 spectrophotometer. EI mass spectrometer from Shimadzu. Conductivity was measured using ELICO conductometer and potentiometry was done using digitech pH meter.

C. Methods of Preparation of Vanadium metal complexes:

a) Vanadyl bis (acetylacetonate), **VO (AA)₂**:

Vanadyl salt ($\text{VO}_2\text{SO}_4 \cdot x \text{H}_2\text{O}$) (0.163g, 1mmol) was dissolved in 5 ml of water. 0.2 ml (2 mmol) of acetyl acetone (2, 4- pentanedione) is added and the solution is stirred for 10 minutes. The solution is neutralized by adding a solution of 2mmol of sodium carbonate dissolved in 10 ml of water slowly with continuous stirring to avoid excessive frothing. The precipitated product is collected by filtration on a Buchner funnel and dried by drawing air through it. Recrystallize the product by dissolving in the minimum amount of hot chloroform (about 4 ml) in a small Erlenmeyer flask,

filtering hot by gravity through fluted filter - paper, cooling to room temperature and adding 10 ml of hot chloroform to complete the precipitation. Filter and allow it to dry in air. Final product obtained is 0.21g. Yield is 80%



Synthesis of above Vanadium complex of acetylacetone is as per literature [38].

b) Vanadyl bis(1,1,1-trifluoro-2,4-pentanedione), **VO(TFAA)₂**

Vanadyl salt ($\text{VOSO}_4 \cdot x\text{H}_2\text{O}$) (0.163g, 1mmol) was dissolved in 10% H_2SO_4 to prevent hydrolysis. Then the ligand (TFAA) in methanol solution was added to the above solution. This solution is neutralized by adding sodium carbonate solution i.e adjusting the pH (1.61) to 4 with alkaline solution with continuous stirring in order to avoid excess frothing. The precipitated product was collected by filtration and dried. Recrystallization of the product was done by using chloroform. The final product was dried over anhydrous calcium chloride. Weight of the final product is 0.24g. Yield is 65%

c) Vanadyl bis(1,1,1-trifluoro-5,5-dimethyl-2,4-hexanedione), **VO(TMTFAA)₂**

Vanadyl salt ($\text{VOSO}_4 \cdot x\text{H}_2\text{O}$) (0.163g, 1mmol) was dissolved in 10% H_2SO_4 to prevent hydrolysis. Then the ligand (TMTFAA) in 5ml of methanol solution was added to the above solution. This solution is neutralized by adding Na_2CO_3 solution i.e adjusting the pH (1.01) to 4 with alkaline solution with continuous stirring at 60 °C. The drab green product was collected by filtration and dried. Recrystallization of the product was done by dissolving in chloroform since sodium bicarbonate and vanadyl hydroxides are insoluble in this solvent. The final product was dried over anhydrous calcium chloride. Product obtained is 0.25g. Yield is 57%

d) Vanadyl bis(1-phenyl-1,3-butanedione), **VO(PhAA)₂**

Vanadyl salt ($\text{VOSO}_4 \cdot x\text{H}_2\text{O}$) (0.163g, 1mmol) was dissolved in 10 ml of H_2O maintained at 78 °C. 5 ml of sodium carbonate (2 mmol) was added to the above solution while stirring i.e adjusting pH 1.69 to 5.95. After 2 min, the ligand (2 mmol) in CH_3OH was added upon which time a green solid was observed. This mixture was

stirred continuously for 1 hr at 78 °C. The precipitated product was collected by filtration on cooling to room temperature. The obtained product was washed with two 3ml portions of H₂O and allowed to dry in air. Final product obtained is 0.253g. Yield= 65%

e) Vanadyl bis(1,3-diphenyl-1,3-propanedione), **VO(DPhAA)₂**

Vanadyl salt (VOSO₄.xH₂O) (0.163g, 1mmol) was dissolved in 50% methanol maintained at 78 °C. 5 ml of sodium carbonate was added to the above solution with stirring maintaining pH 6. After 2 min, ligand (2 mmol) was added upon which a dark green solid was observed. This mixture was stirred continuously for 1 hr at 78 °C. The final product was collected by filtration on cooling to room temperature. The obtained product was washed with two 5 ml portions of chloroform since sodium bicarbonate and vanadyl hydroxides are insoluble in this solvent and the final precipitate was dried over calcium chloride. Yield=55%, product obtained was 0.28g.

f) Vanadyl bis(4,4,4-trifluoro-1-phenyl-1,3-butanedione), **VO(TPhAA)₂**

To the solution of Vanadyl sulphate (0.163g, 1mmol) in 5 ml of H₂O maintained at ~ 78 °C was added 5 ml of sodium acetate (2 mmol) with rigorous stirring which lead to a color change from blue to dark blue green (pH 5 was maintained). After some time, TPhAA (2 mmol) was added and stirred for 30 min maintaining 78 °C which resulted a drab yellow solid. Cool the final product to room temperature and filter it with H₂O and recrystallize with methanol and dried over P₄O₁₀. Final product obtained is 0.34g. Percentage yield is 69%

g) Vanadyl bis(2,2,6,6-tetramethyl-3,5-heptanedione), **VO(TMh)₂**

Vanadyl salt (VOSO₄.xH₂O) (0.163g, 1mmol) was dissolved in 50% methanol maintained at 78 °C. 5 ml of sodium acetate was added to the above solution with stirring. The ligand (2 mmol) solution dissolved in CH₃OH was added upon which a green solid was observed. This mixture was stirred continuously for 1 hr at 78 °C. The final product was collected by filtration on cooling to room temperature. The obtained product was washed with two 5 ml portions of water and dried over calcium chloride. Yield=39%, weight of the final product is 0.16 g.

h) Vanadyl bis(4,4,4-trifluoro-1,2-thienyl-1,3-butanedione), **VO(TTA)₂**

To a solution of Vanadyl sulphate (0.163g, 1mmol) in 5 ml of H₂O maintained at ~ 78 °C was added 5 ml of sodium acetate (2 mmol) with rigorous stirring which lead to a color change from blue to dark blue green while maintaining pH 5. After some time, TTA (2 mmol) was added and stirred for 30 min maintaining 78 °C which resulted a orange yellow solid. Allow the compound to cool down to room temperature and filter it with H₂O and recrystallize with chloroform and dried over P₄O₁₀. Final product obtained is 0.27 g. Yield = 52%

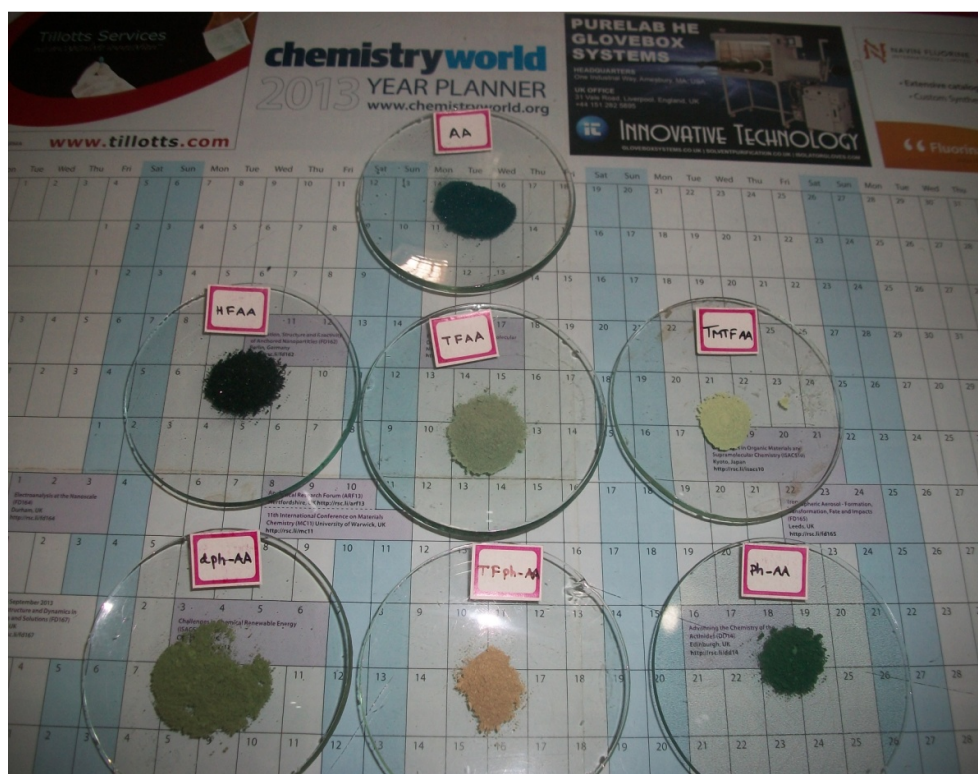


Fig 3: Few synthesized β -diketone containing Vanadium (IV) complexes.

D. Procedures for characterization:

i. *UV-Vis spectroscopy:*

Standard solutions (2×10^{-3} M) of all vanadium complexes were prepared in methanol and DMSO. 2ml of each vanadium solution was taken in cuvette and the spectrum was recorded. The wavelength at which maximum absorbance occurred was noted down.

ii. *IR spectroscopy:*

IR spectra of all complexes were recorded using KBr pellets.

iii. *Conductometry:*

Standard solutions (2×10^{-3} M) of all vanadium complexes were prepared in 100ml CH_3OH . The cell was calibrated by 0.01 M KCl (1.413 mS cm^{-1}). The conductance of all complexes were measured. All the measurements were done at $25 \pm 0.1^\circ \text{C}$.

iv. *^1H NMR:*

All the samples were dissolved in CDCl_3 , placed in the NMR tubes and recorded. Mestrek software was used for obtaining the spectra.

E. Procedure for Solution studies (Potentiometry):

i. *Estimation of Vanadium (IV):*

Vanadium (IV) salt was estimated by KMnO_4 because hydrated salts have varied concentrations.



ii. *Preparation of standard solutions for pH titrations:*

0.01M of metal solution (100 ml), 0.1N KNO_3 (100 ml) solution, 0.05N NaOH was prepared. 0.01M ligand solution (100 ml) was prepared. 5ml of each solution except NaOH was added into the reaction cell and the solution was made upto 50 ml by adding water. So, the final concentration of the solution will be 0.001 M. The above

solution was titrated against 0.05 NaOH. 0.1 M KNO_3 was added to maintain ionic strength. All the titrations were performed at $25 \pm 0.1^\circ \text{C}$.

F. Procedure for Dialysis:

Dialysis bag was soaked in hot water bath till both the ends are opened. One side of the tubing bag was tied with a thread and the sample (2ml of 5 mg/ml BSA (M.wt:66,000) and 2ml of 0.037 M complex was added) was loaded into the bag and the another side of the bag was closed (with thread). The sample was placed into a 500 ml beaker containing 0.1 M PBS buffer (pH 7.2). The apparatus was kept overnight (providing infinite time to the sample) with continuous stirring.

IV. Results

Solubility tests were conducted. The results revealed their solubility in different polar and non-polar organic solvents. All complexes were soluble in methanol, chloroform, DMF, DMSO. Phenyl substituted complexes were soluble in benzene as well. The data is provided in the following table.

Table 2: Solubility of complexes

complex	H ₂ O	CH ₃ OH	CHCl ₃	DMF	DMSO	Ether	C ₆ H ₆
VO(AA) ₂	✓	✓	✓	✓	✓	✓	✗
VO(TFAA) ₂	✗	✓	✓	✓	✓	✓	✗
VO(TMTFAA) ₂	✗	✓	✓	✓	✓	✓	✗
VO(PhAA) ₂	✗	✓	✓	✓	✓	✓	✓
VO(DPhAA) ₂	✗	✓	✓	✓	✓	✓	✓
VO(TFPhAA) ₂	✗	✓	✓	✓	✓	✓	✓
VO(TTA) ₂	✗	✓	✓	✓	✓	✓	✓
VO(TMH) ₂	✗	✓	✓	✓	✓	✓	✗

Decomposition temperature was measured using melting point apparatus. The conductivity of complexes of 0.002 M concentration dissolved in CH₃OH. Magnetic moments of all the complexes are performed using Gouy balance.

Table 3: Physical data of complexes

Complex	Color	Yield (%)	Decomposition Temp. (°C)	μ _{eff} (BM) 300 K	Λ _m (Scm ² mol ⁻¹)	λ(max)
VO(AA) ₂	Bluish green	80	180	1.74	0.2	250
VO(TFAA) ₂	Green	65	190	1.76	0.4	296
VO(TMTFAA) ₂	Olive green	57	200	1.74	0.12	310
VO(PhAA) ₂	Green	65	218	1.70	0.414	326
VO(DPhAA) ₂	Green	55	230	1.68	0.312	350
VO(TFPhAA) ₂	Yellow	69	226	1.73	0.08	336
VO(TMH) ₂	Green	39	220	1.76	0.27	313
VO(TTA) ₂	mustard	52	230	1.75	0.07	352

Table 4: Analytical results of synthesized complexes

Comp	Empirical formula	M.Wt	V%	C%	H%	O%	F%	S%
a	C ₁₀ H ₁₄ O ₅ V	265.15	19.22 (19.21)	45.31 (45.29)	5.33 (5.32)	30.18 (30.16)		
b	C ₁₀ H ₈ F ₆ O ₅ V	373.10	13.68 (13.65)	32.19 (32.19)	2.18 (2.16)	21.42 (21.44)	30.552 (30.55)	
c	C ₁₆ H ₁₄ O ₅ F ₆ V	451.22	11.30 (11.28)	42.60 (42.59)	3.12 (3.13)	17.731 (17.29)	25.26 (25.26)	
d	C ₂₀ H ₁₈ O ₅ V	389.30	13.10 (13.08)	61.71 (61.70)	4.66 (4.66)	20.55 (20.55)		
e	C ₃₀ H ₂₂ O ₅ V	513.44	9.92 (9.92)	70.18 (70.17)	4.32 (4.31)	15.60 (15.58)		
f	C ₂₀ H ₁₂ F ₆ O ₅ V	497.24	10.25 (10.24)	48.30 (48.30)	2.435 (2.43)	16.08 (16.09)	22.93 (22.92)	
g	C ₂₂ H ₃₉ O ₅ V	434.48	11.74 (11.72)	60.82 (60.82)	9.06 (9.05)	18.40 (18.41)		
h	C ₁₆ H ₉ F ₆ O ₅ S ₂ V	510.30	9.98 (9.98)	37.68 (37.66)	1.78 (1.78)	15.68 (15.69)	12.58 (12.57)	22.32 (22.34)

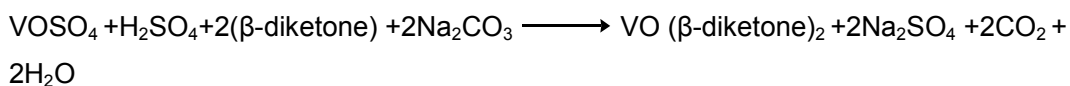
Table 5: Band assignments of FT-IR spectra of vanadium complexes

Complex	ν (V=O)	ν (C-O)	ν (C-C)	ν (V-O)
VO(AA) ₂	996.57	1560,1525.84	1285.76,1360	483.39
VO(TFAA) ₂	928.26	1534.60	1293.98	446.68,535.42
VO(TMTFAA) ₂	938.076	1547.492	1309.3	495.47,534.08
VO(PhAA) ₂	995.50	1548.9	1206.02	450.12
VO(DPhAA) ₂	993.01	1529.457	1226.34	462.37
VO(TFPhAA) ₂	946.8	1570	1290	448.512
VO(TMH) ₂	966.34	1570,1539.20	1286	531.5
VO(TTA) ₂	939.33	1597,1575.84,1543	1352,1298.09	527

V. Discussion:

A. Synthesis and characterization of synthesized complexes:

All the complexes are synthesized in good yields. The general reaction for a, b, c, d, e is given below:



Equation for f, g and h is

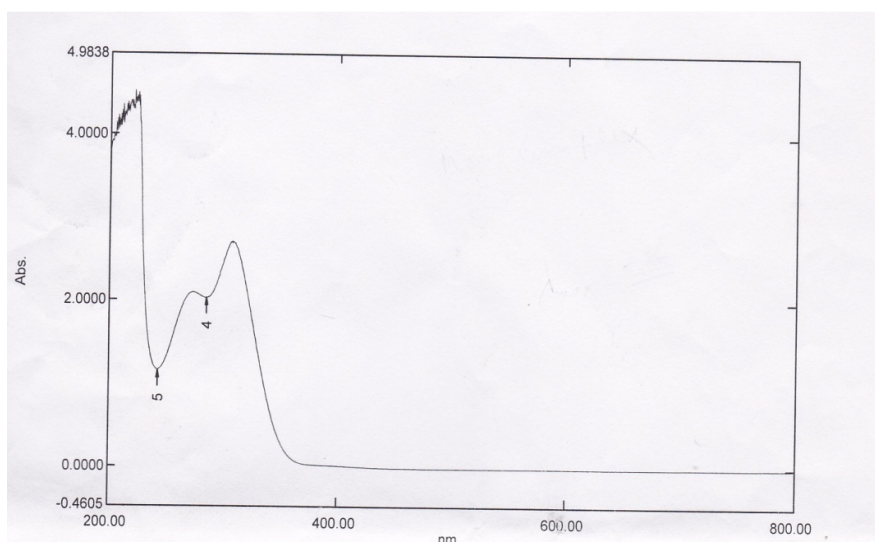
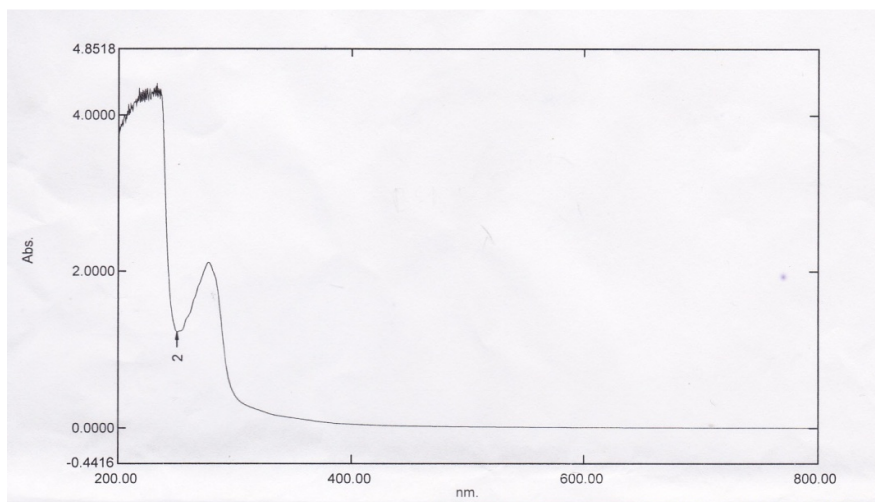


The molar conductance of complexes were obtained in the range 0.05- 0.5 S cm² mol⁻¹ dissolved in CH₃OH which indicated them as non-electrolytes [39] (data is given in the table 3). CH₃OH does not participate in the reaction since it is a molecular compound which do not dissociate into ions. So, they do not show conductivity (behave as non-electrolytes). Magnetic moments of all the complexes are in the range of 1.68-1.76 BM corresponding to one lone pair of electrons (d¹ configuration of V).

UV-Vis spectra:

All the complexes exhibited four transitions in UV and visible regions in DMSO. The peaks appeared in the visible region are due to the d-d transitions of metal and the peak observed in the UV region is due to $\pi\text{-}\pi^*$ transition of the ligands. The spectrum is shown below.

Fig 4: UV-Vis spectrum of $\text{VO}(\text{AA})_2$ and $\text{VO}(\text{TFAA})_2$



FT-IR spectra:

All the band assignments were done by comparing the spectral data of free ligands and the complexes and were based on the vibrational assignment of $\text{VO}(\text{AA})_2$ [40] (refer table 5). The characteristic $\text{V}=\text{O}$ stretching mode was observed at $920\text{--}940\text{ cm}^{-1}$ for fluoro-substituted β -diketones which was much lower than $\text{VO}(\text{AA})_2$. This is due to the effect of neighboring species which was well known[41] i.e the electron withdrawing groups attached to the β -diketone accounted for lower frequency shift. No band was observed around 1610 cm^{-1} region indicating C-O group of β -diketones was not free in the complexes implying metal chelation to the C-O group. Diketones exhibited two bands, one at 1700 cm^{-1} and other at 1725 cm^{-1} , upon chelation they were disappeared. Absorption bands were observed below 700 cm^{-1} in all the complexes which are caused by metal-oxygen (V-O) vibrations. The carbon double bonds have less double bond character and more single bond character in the conjugated chelate ring showing a large frequency shifts of C=C absorption band and carbonyl band after chelation. It is expected that if more conjugation between vanadium ion and carbonyl ion exists, then a stronger metal-oxygen bond is seen which resulted in lowering of frequency of the perturbed carbonyl band [42]. The spectral assignments showed comparable results with $\text{VO}(\text{AA})_2$. The vibrational pattern of the prepared complexes with uncoordinated ligands explains that metal coordination does not affect the principal internal modes of the R and CF_3 fragments. The assessment of frequencies of mentioned most relevant vibrational nodes (refer to table 5) revealed a close similarity with the parent complex which suggested that the synthesized complexes share square pyramidal geometry. Refer figures 5-12 for spectral details.

Fig.5 IR spectra of VO(AA)₂

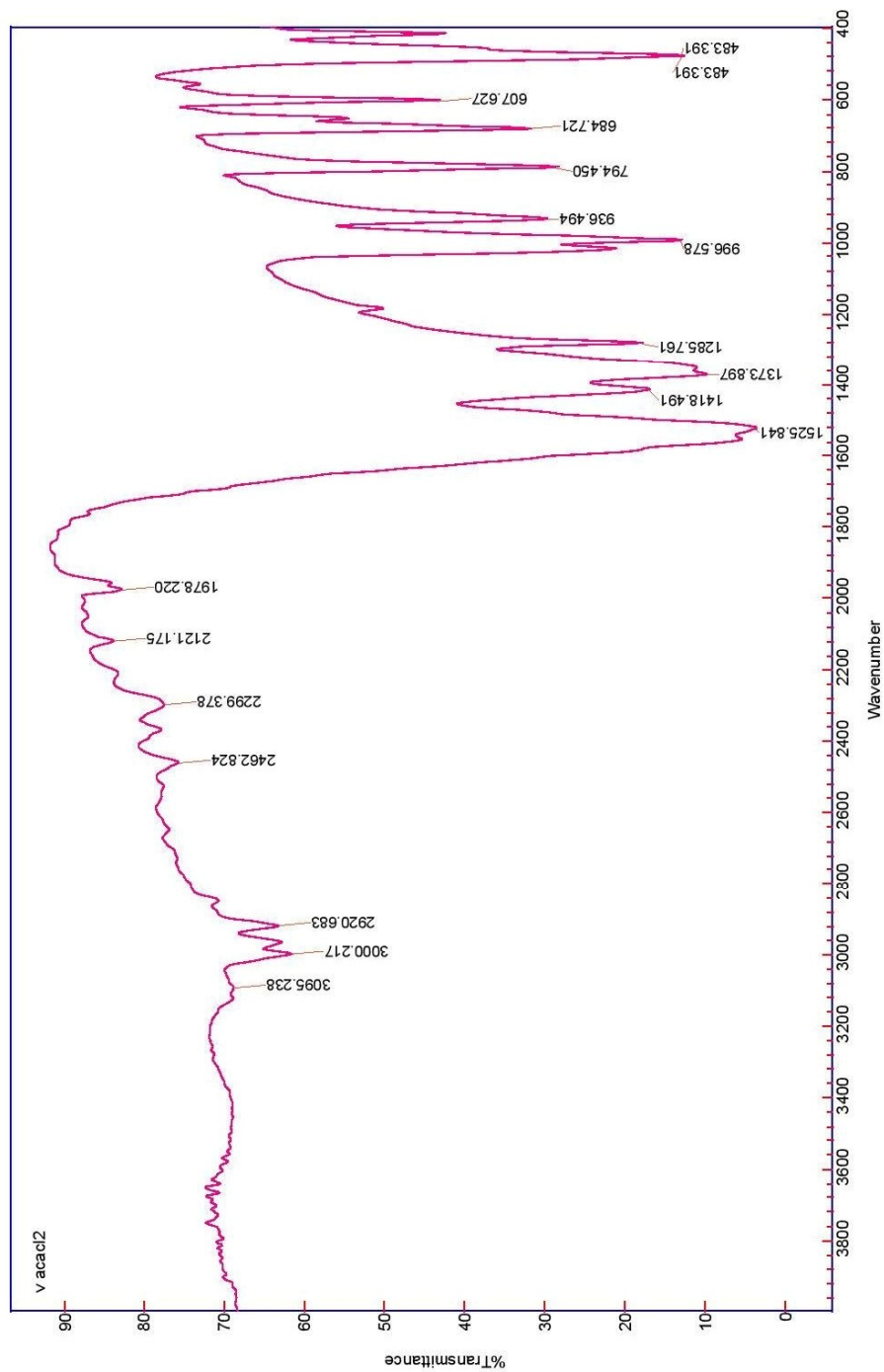


Fig 6: IR spectra of VO (TFAA)₂

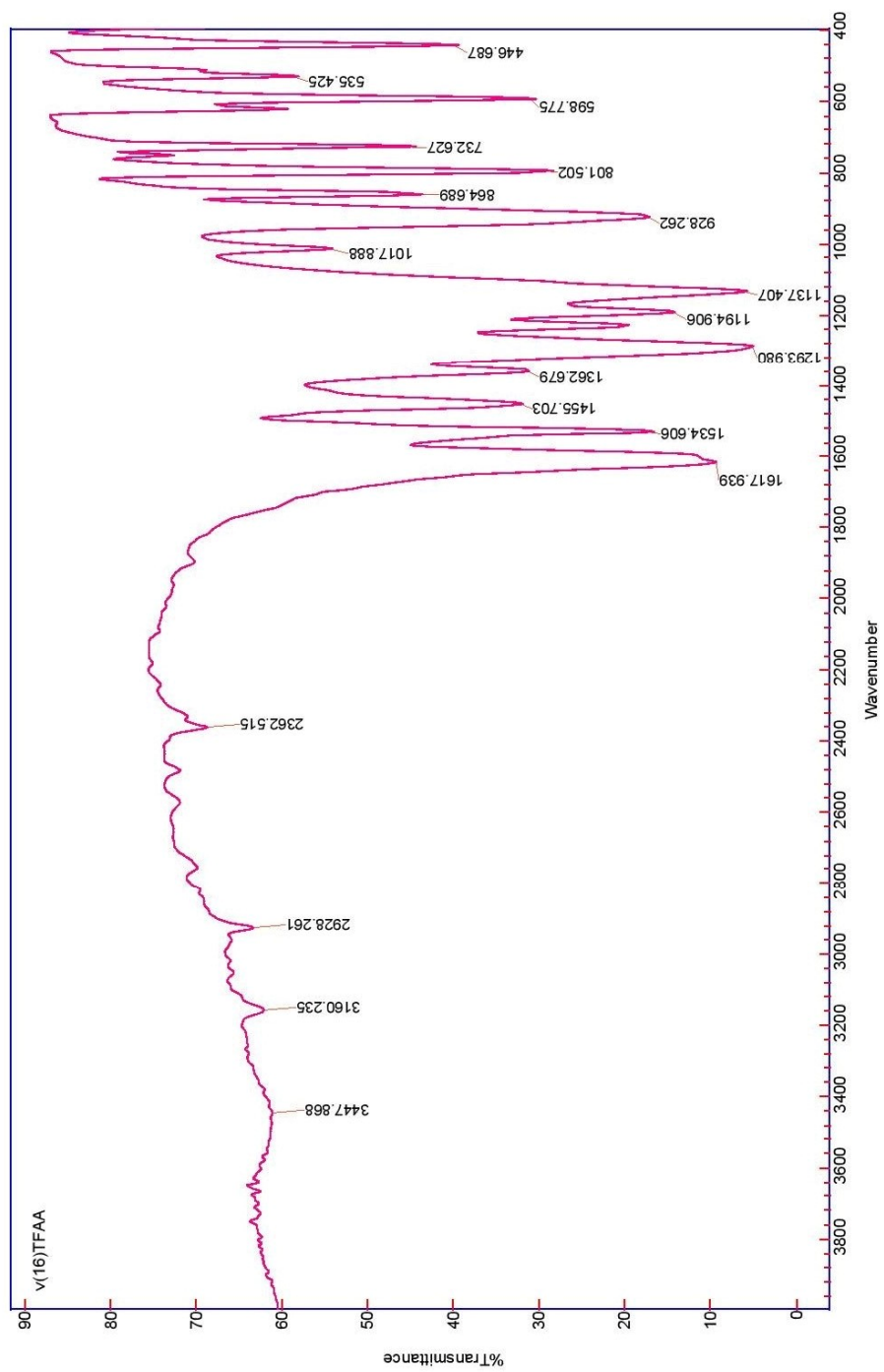


Fig 7: IR spectra of VO(TMTFAA)₂

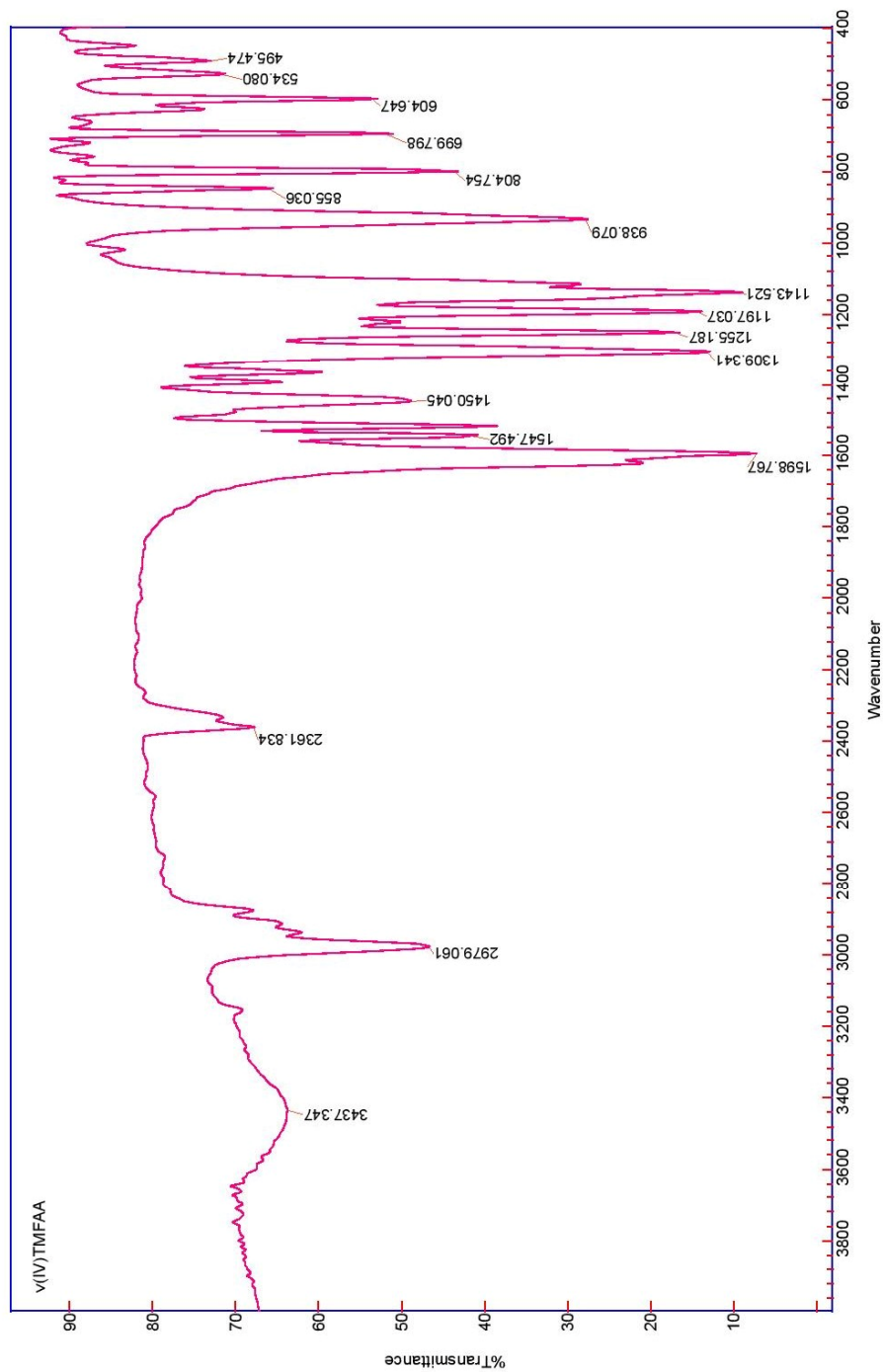


Fig 8: IR spectra of VO(PhAA)₂

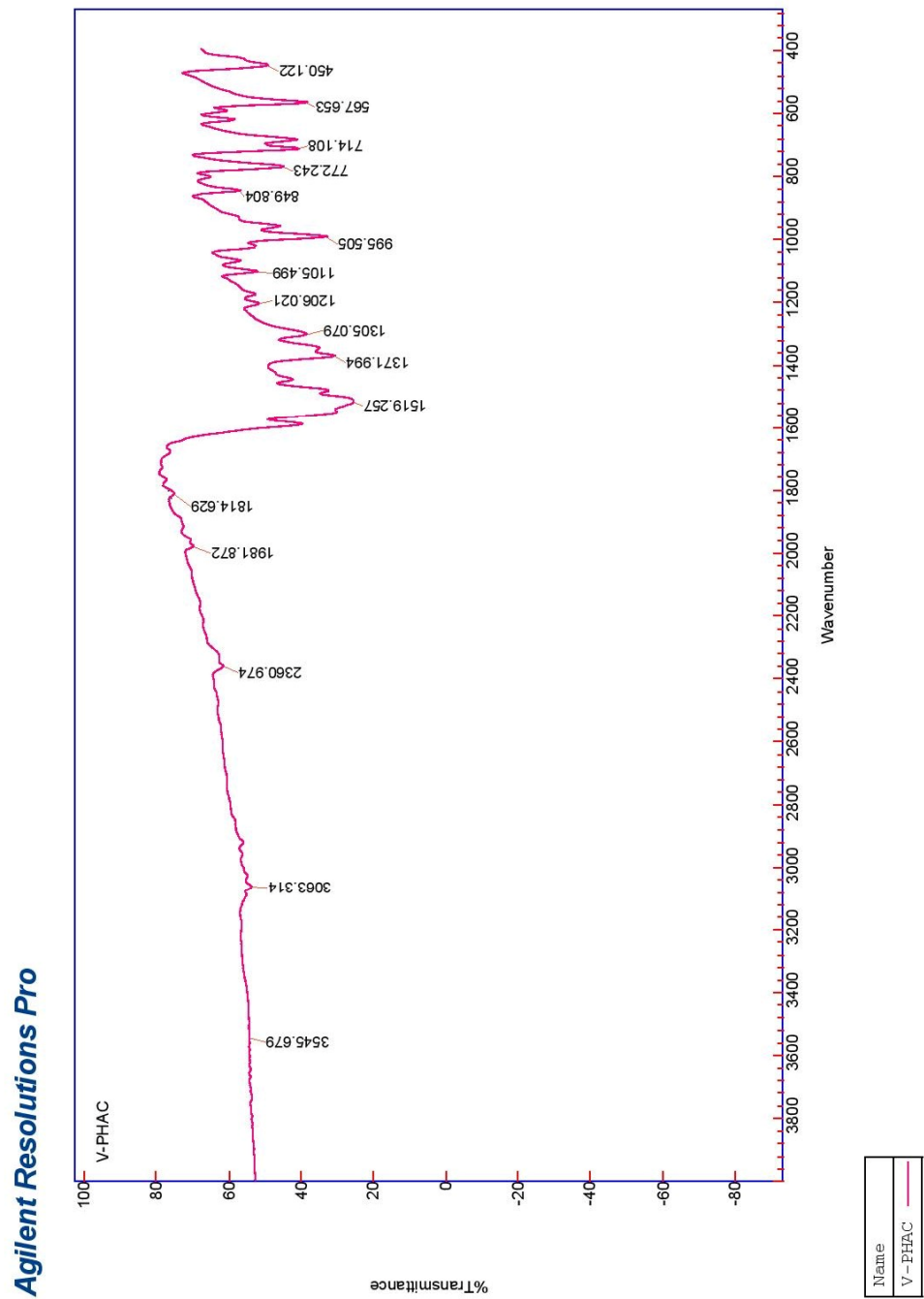


Fig 9: IR spectra of VO(DPhAA)₂

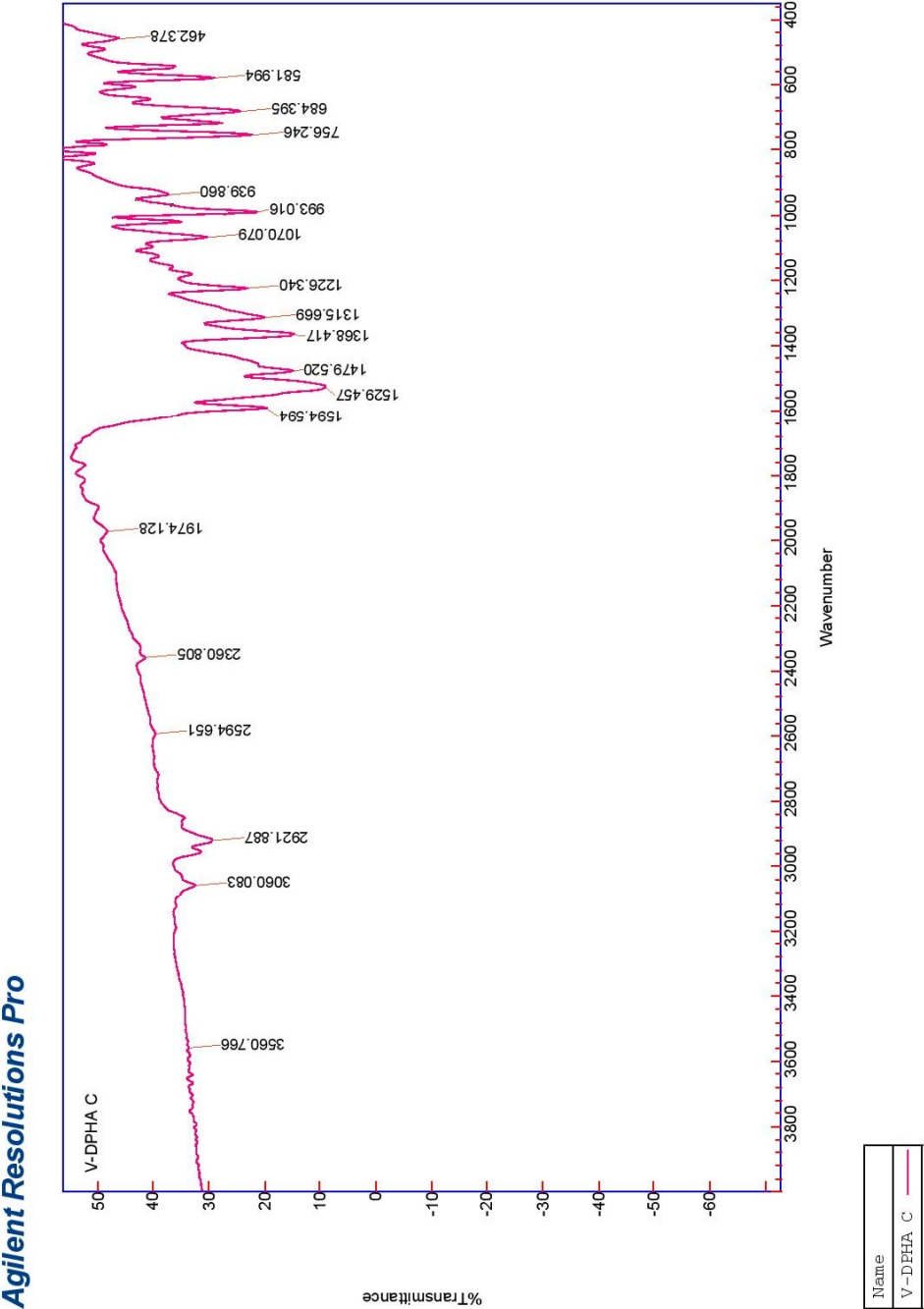


Fig 10: IR spectra of VO(TFPhAA)₂

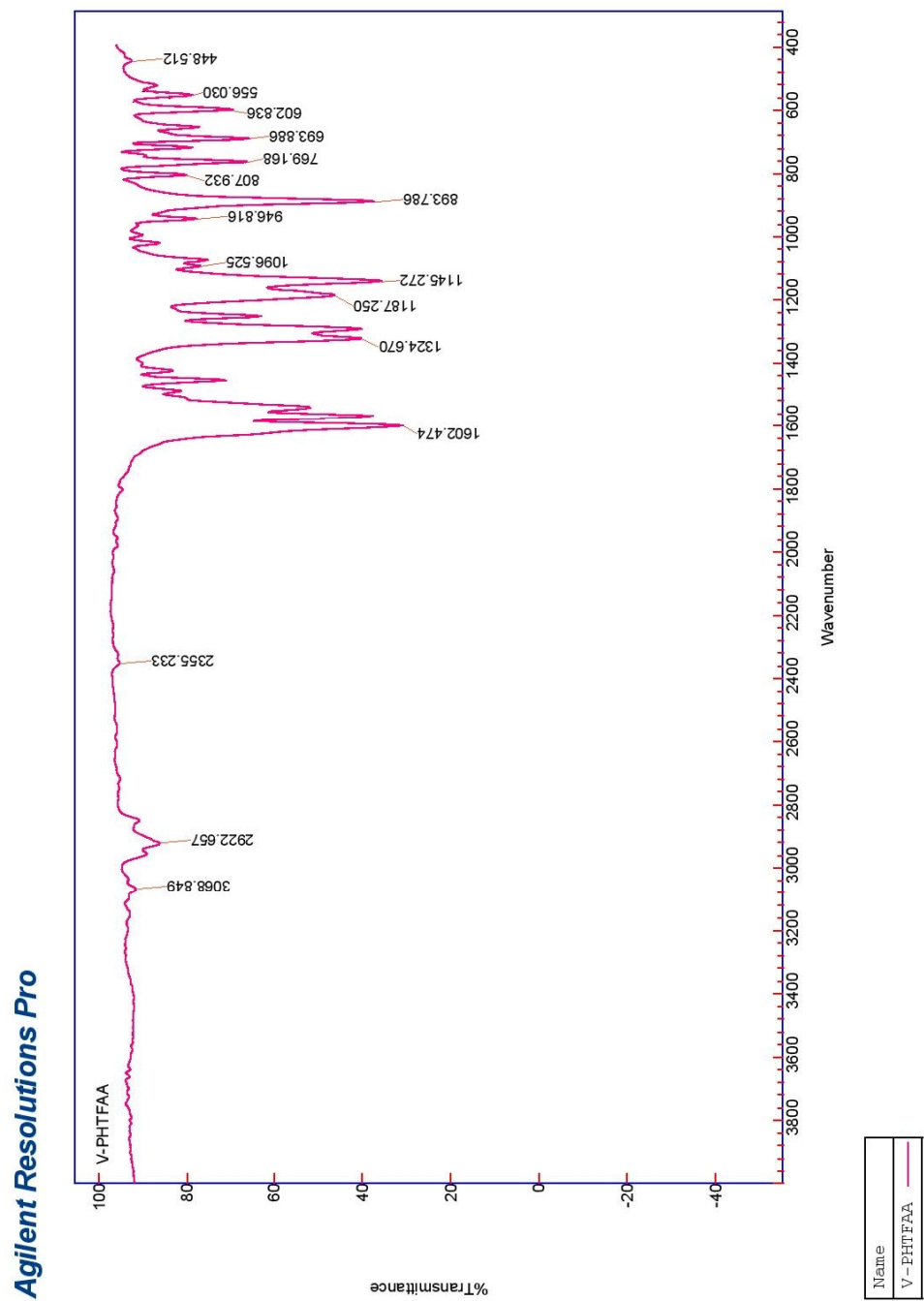


Fig 11: IR spectra of $\text{VO}(\text{TMH})_2$

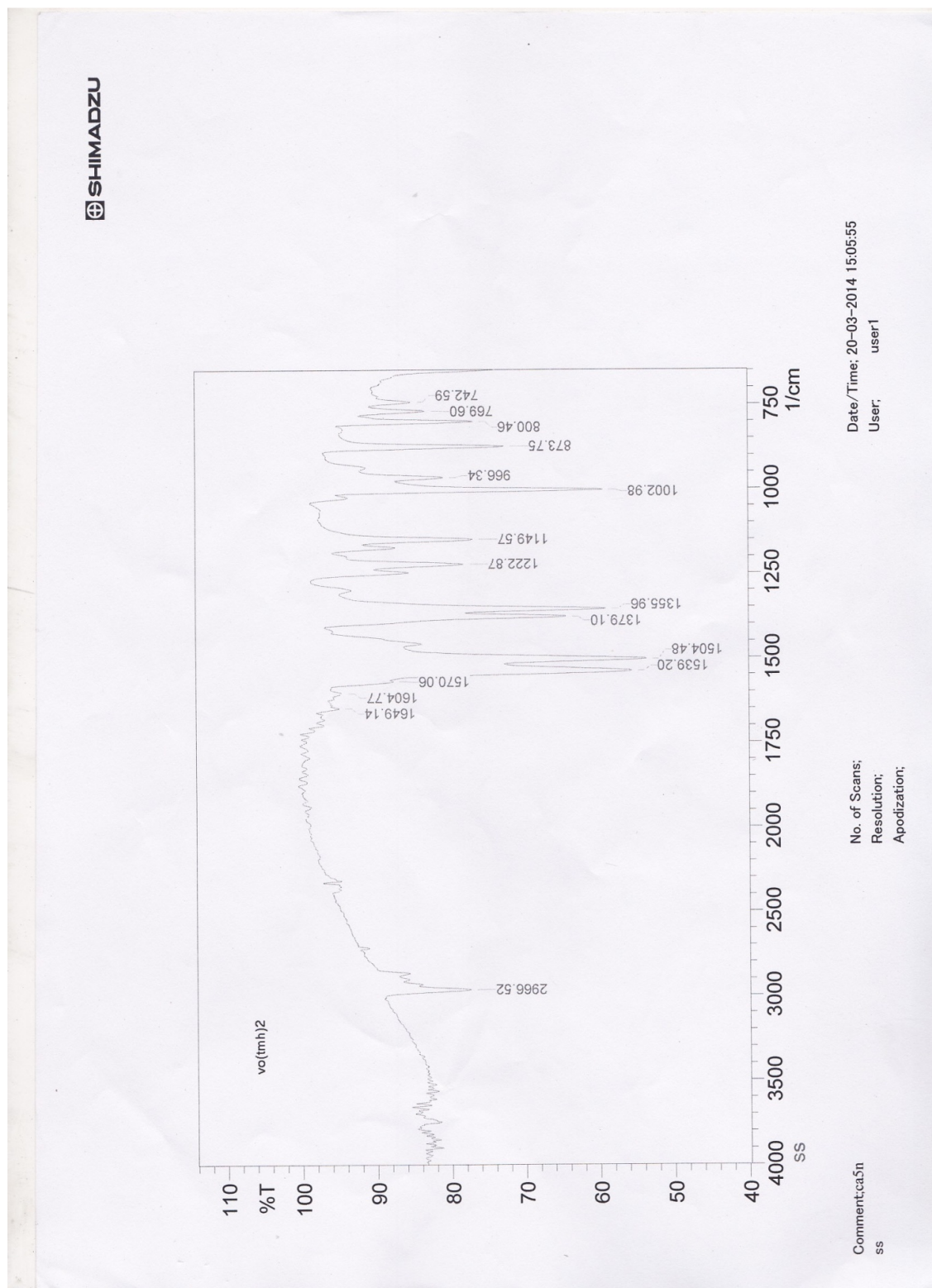
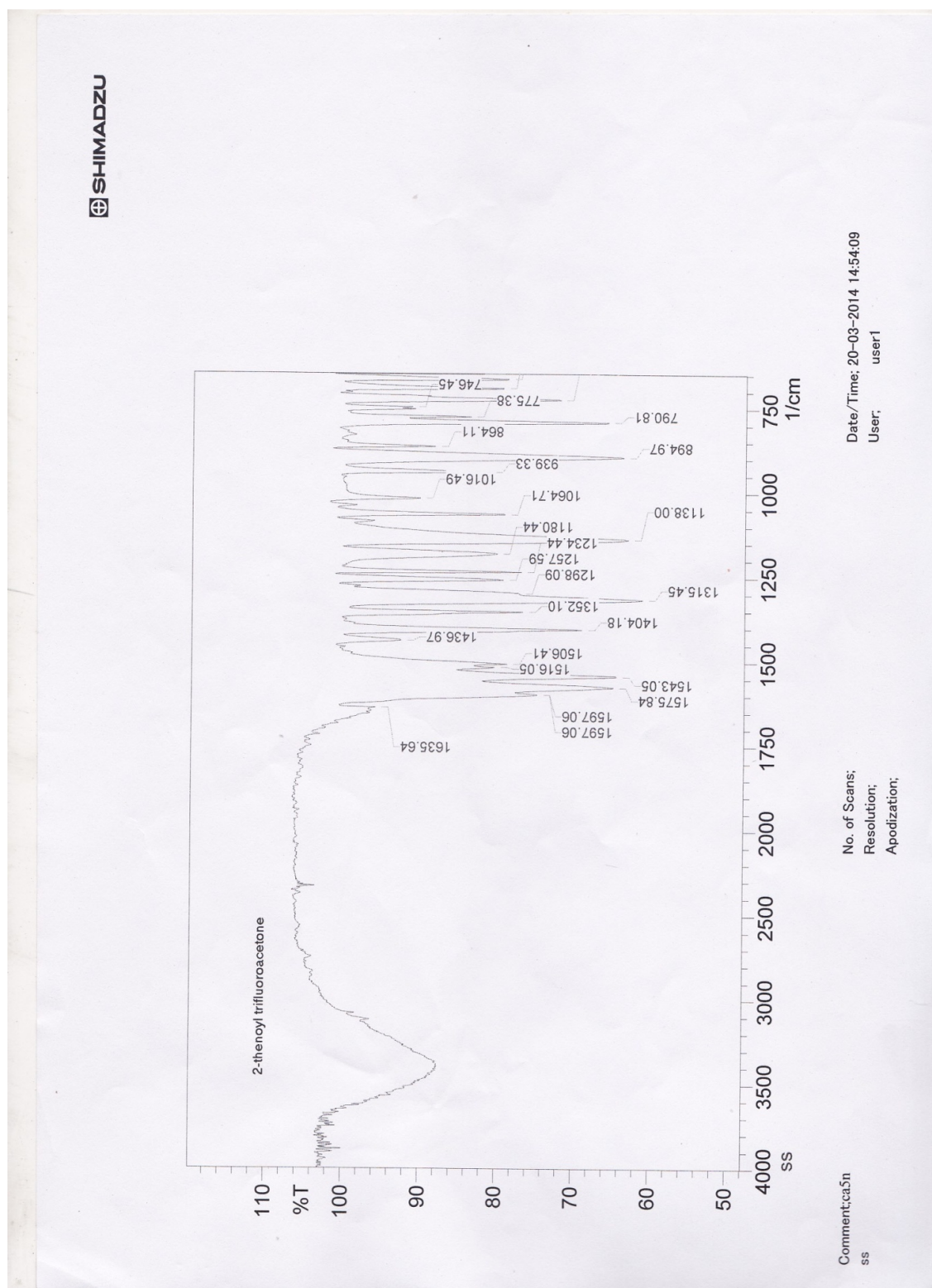


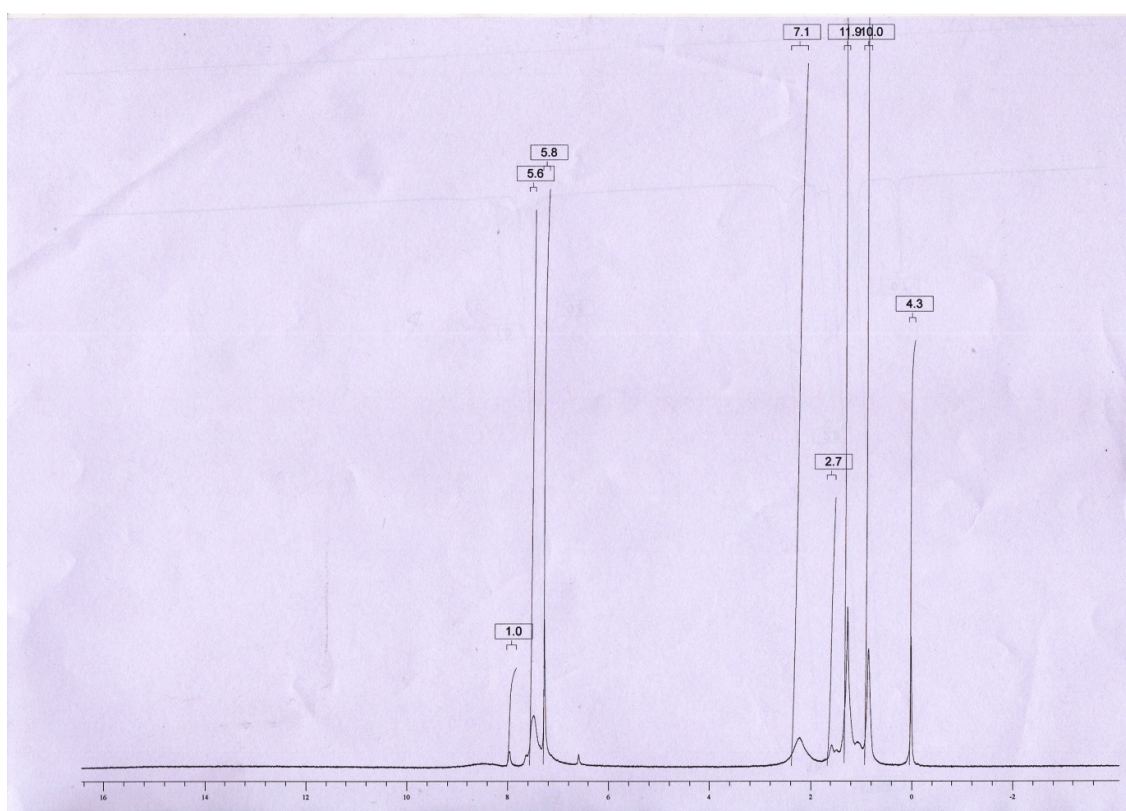
Fig 12: IR spectra of $\text{VO}(\text{TTA})_2$



^1H NMR:

Although Vanadium is paramagnetic and proton NMR cannot be obtained but attempt was made to observe the removal of OH proton. No signal was seen in the region 11.0-10.0 ppm which revealed that deprotonation occurred and bonding of β - diketones to vanadium metal ion (refer fig.13). The paramagnetic nature of vanadyl ion affected the ligand resonances which exhibit line broadening which can be taken as an evidence of complexation [43].

Fig 13: ^1H NMR spectra of $\text{VO}(\text{DPhAA})_2$



B. Solution studies

Solution studies were performed using pH metry.

All the solutions were prepared using freshly boiled, degassed, deionised water to ensure the removal of dissolved oxygen and CO_2 . Firstly, the metal to ligand ratio maintained was 1:1. The problems faced during the titrations are

- i) At pH more than 4, the color of the complex solution was changed from blue to yellow.
- ii) At pH above 6, there are fluctuations in the pH values (unstable values) and precipitate formation was observed.

So, It was expected that vanadyl (+4) ion was more susceptible to vanadate ion (+5) i.e oxidation took place at pH more than 4. Formation of insoluble hydroxides were observed which implied hydrolysis at pH above 6. Then, metal to ligand ratio was kept in 1:5, 1:10. But the oxidation problem was not eradicated. To neutralize that, 0.01 M acid was added. The pH values were stable until volume was reached to 1 ml but the values got fluctuated for higher volumes.

This study was discontinued due to lack of time.

In few theses, it was mentioned that all the titrations must be performed with continuous flow of purified nitrogen using HCl and TMAOH (tetramethyl ammonium hydroxide which is a weak base). The ionic strength was maintained at 0.1 M TMACl.

C. Binding studies of complexes with BSA:

When the vanadium complex enters the body, it may undergo transformations in the gastrointestinal tract through circulation and in tissues and cells finally [44-47]. In few papers, it was discussed that the complex undergoes rapid dissociation at the site of absorption furthermore the ligand and the vanadyl ion follow diverse pharmacokinetic pathways [44, 48, 49]. Preliminary study was done to check the % of absorption of vanadium complex with BSA over time. All the vanadium complexes of substituted β -diketones showed comparable binding properties with respect to the parent complex. The interaction of vanadyl(IV) ions with BSA was shown in the figure 14, 15. The UV-Vis spectra of BSA exhibited two peaks around 200 and 280 nm assigned to peptide bond and aromatic amino acid side chains respectively. UV-Vis spectra of BSA+VO⁺² complex displayed a considerable increment in the intensity around 280 nm band. Furthermore, it was proposed that strong interactions between vanadyl cation and bovine serum albumin would be anticipated at pH 7. Not much was studied about the structural and conformational modifications in the protein after the metal chelation.

Fig 14: Absorption spectra of VO(AA)₂

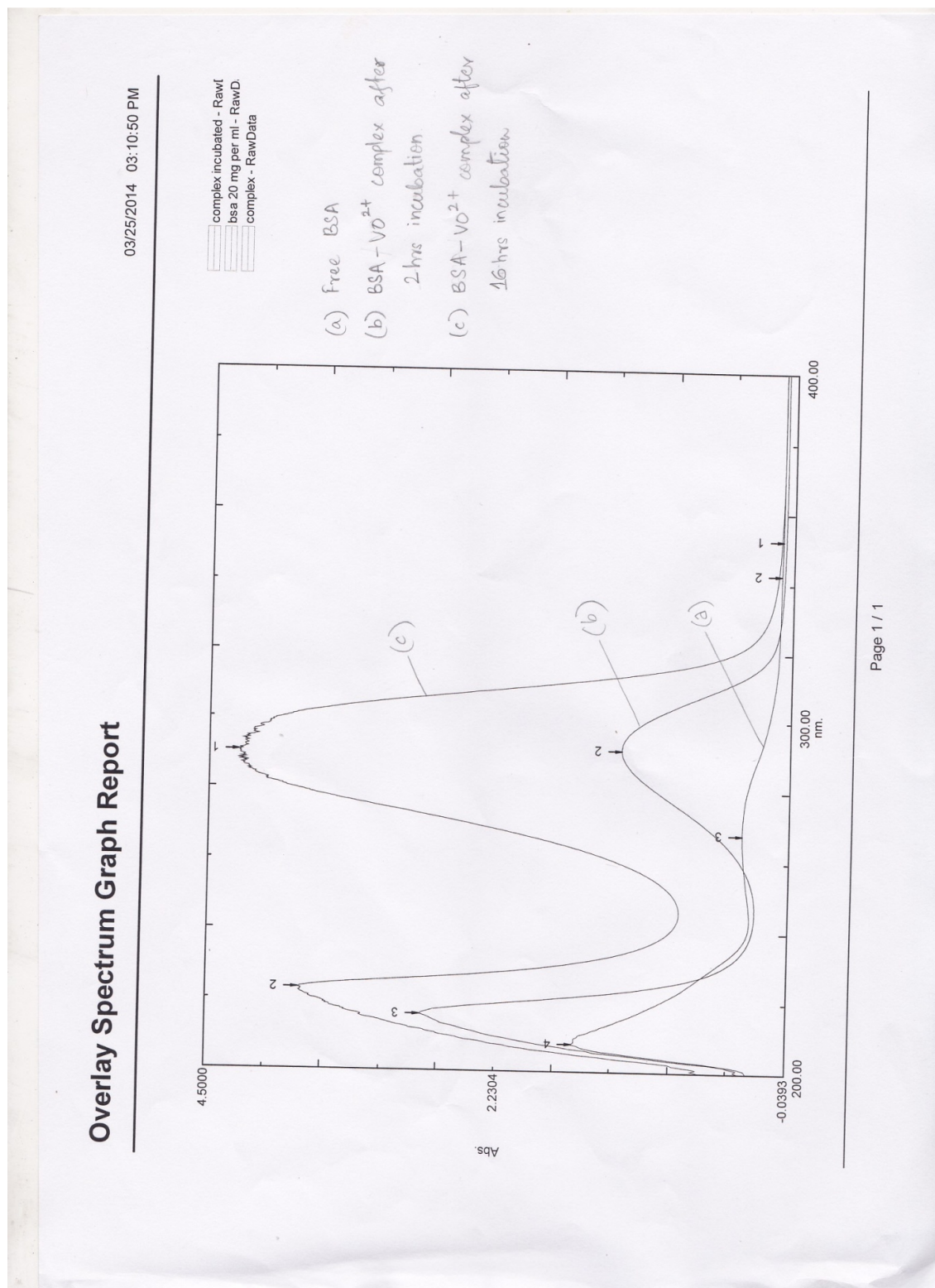
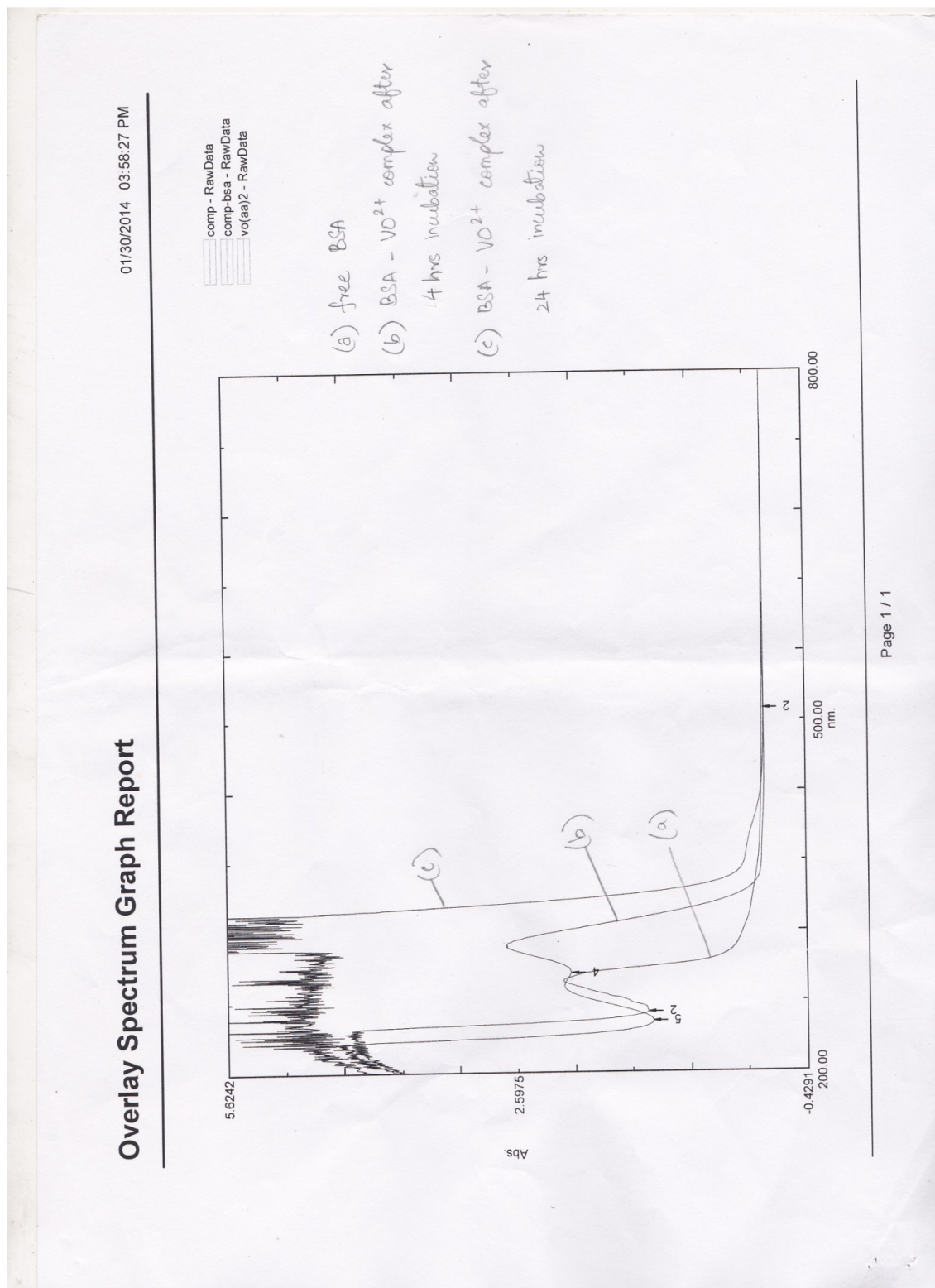


Fig 15: Absorption spectra of $\text{VO}(\text{TFAA})_2$



VI. Conclusions

Vanadyl complexes have been synthesized, characterized and interactions with BSA are investigated in brief. All the synthesized complexes showed comparable results with parent complex, VO (AA)₂. All the spectral studies revealed the coordination of vanadyl ion to the ligand (β -diketone). It was evidenced that the complexes attained square pyramidal geometry. Thorough study is still needed to assess the structure and the stability of the complexes. The results obtained in the absorption studies helped to afford more evidences on the binding properties of vanadium species with BSA. Further investigation was necessary because all the findings in binding studies may facilitate for development of new therapeutic agents for the treatment of Type II DM since protein- chelate interactions are associated with enhancement of their insulin mimetic activity.

VII. References

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