Journal of Basic and Applied Engineering Research
p-ISSN: 2350-0077; e-ISSN: 2350-0255; Volume 4, Issue 7; October-December, 2017, pp. 630-639

http://www.krishisanskriti.org/Publication.html

# Spectrophotomeric Determination of Antibiotic Drug Penicillin via its Quantitative Conversion to Pencillamine and its Complexation with OS (VIII): Characterization of Complex by FTIR, NMIR, ESR, TGA, DTA. Proposed Structure of the Complex

**Kavita Pal Sharma**

*[1]Department of Chemistry, [1]Hawabagh Women's College, Jabalpur, M.P. INDIA*

**Abstract**—Micro determination of the well established antibiotic drug penicillin has been developed by its quantitative conversion to penicillamine. It involves stepwise complexation with Os(VIII) forming 1:1 complex, with λ max = 238nm. E =6.70x$10^2$ L $mol^{-1}$ $cm^{-1}$, Beer's law range 1.2600x$10^{-1}$ mg to 3.192mg. % error is less than 1.0%, standard deviation 1.012x$10^{-4}$ and coefficient of variance 0.0080. Effect of foreign metal ion shows that 1 ppm of complex could tolerate 0.000056 ppm of Fe(II), 0.0372 ppm of Fe(III), 0.002 ppm of Cu(II), 0.0056 ppm of Co(II), 0.0005 ppm of Ni(II), where as Palladium(II) and Ru (III) cause severe interference. Characterization of the complex was done by elemental analysis.(CHN), which confirms 1:1 ratio between metal and ligand. FTIR spectrum of the complex also confirms the binding of –SH and $NH_2$ Group to Os(VIII) by disappearance of –SH stretch art (2519 $cm^{-1}$) and C-N stretch (at 1354$cm^{-1}$) in the Os(VIII) complex. Since no ESR signals were obtained by complex hence it shows diamagnetic nature as well as square bipyramidal geometry which is confirmed $^1$H NMR spectrum of the complex exhibits down field shift (-0.26) for – SH proton and $NH_2$ protons (10 fold decrease) showing the bonding between Os(VIII) and 'S' atom of –SH group and N of $NH_2$ group. DTA and TGA of this complex have very significantly confirm the proposed structure and presence of water molecules.

**Keywords*: FTIR, NMR, ESR, DSC, TGA & DTA.***

## 1. INTRODUCTION

Antibiotics are chemical substances produced by various species of microorganisms (bacteria, fungi, actinomycetes) that suppress the growth of harmful microorganisms and may eventually destroy them. Antibiotics commonly include synthetic antibacterial agents having selective toxicity for bacterial cells. These includes sulfonamides and Quinolones that are not the products of microbes[1]. The number of antibiotics that has been identified now extends into the hundred and many of these have been developed to the stage where they are of value in the therapy of infectious diseases. About 3000 antibiotics are known at present, of which about 300 have a chemotherapeutic action[2] too. On the basis of different biochemical target in the cell, antibiotic drugs can be divided[3] into following four major classes:

1. Drugs that prevent the synthesis of folic acid, necessary for all cells, e.g. sulphonamides.
2. Drugs that attack the peptide bond during transpeptidation thus affecting the bacterial cell wall formation, e.g. Penicillin.
3. One of the drugs that inhibits the protein synthesis in bacteria by binding it to a protein of unknown function in the ribosome, is streptomycin.
4. Drug that kills bacterial cell by penetrating the cell membrance and disrupting the electrostatic bonded area and ultimately lead to cell's subsequent degradation, e.g. Tyrociding A.

Penicillin, which is an important antibiotic drug, gets hydrolysed in aqueous media and a variety of products are formed depending upon the reaction conditions.

Unlike other groups of natural compounds i.e. alkaloids and glycosides, the antibiotics have no group reaction for their qualitative identification. Thus, identification has to be based on the chemical structure of the particular antibiotic. It is

therefore, the functional groups, which gives distinguishing reactions especially forming coloured products which can then be identified by a number of spectroscopic methods.

A variety of biological, chemical and physiochemical methods are employed for the quantitative determination of antibiotic or antimicrobial drugs. Biological methods are mostly time consuming and the accuracy of the analysis depend on many external factors.

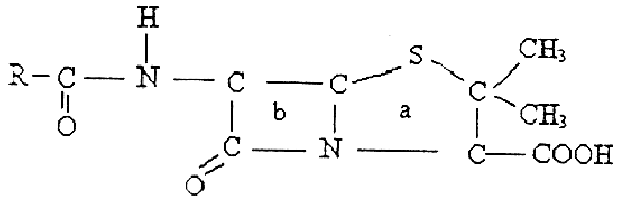
Recent chemical methods for the quantitative analysis of antibiotics preferentially includes photocolorimetry and spectrophotometry[2].

Various methods of assay includes ultraviolet spectrophotometry[4], visible spectrophotometry[5-9], fluorimetry[10], Atomic absorption spectrometry[11], polarography[12], coulometry[13], paper chromatography[14], thin layer chromatography[15], gas-liquid chromatography[16], high performance liquid chromatography[17], titrimetry[18,19] and various inorganic[20-47] and organic methods[48-66] .

The British Pharmacopoeia and United States Pharmacopoeia report a bromimetric and a nitrimetric titration, respectively. However, both the methods are almost equally time consuming.

Penicillin is the first antibiotic used in man. In basic media penicillamine is formed as a quantitative product which is itself an antibiotic drug penicillin interferes with synthesis of bacterial cell wall which consist of mucopetides. This drug attacks he peptide bond at two places. The bond of the D-alanine splits during transpeptidation.

The structure of penicillin consists of β-lactam and thiazolidine ring. The thiazolidine is formed from L-cysteine and D-valine.




a = Thiazolidine b= β lactam

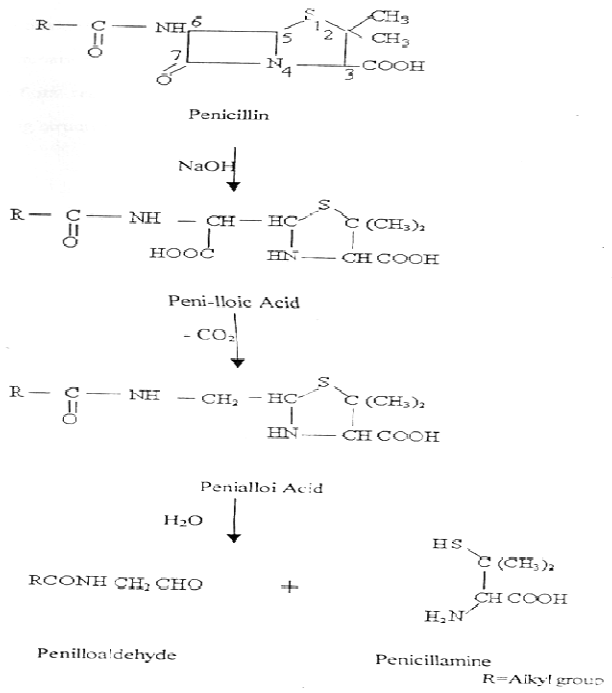
B lactam ring of Penicillin possesses O=C=N bond, which is in the same position as that of the peptide bond in D-Ala-D-Ala, owing to this reason, penicillin molecule is capable of inhibiting the transpeptidase enzyme, by dividing into it active site.

Various methods have been proposed for the quantitative analysis of penicillin. Iodimetric determination of penicillin involves its alkaline hydrolysis and then determining at a pH near 4.5.

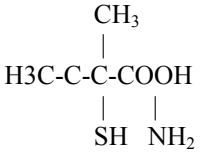
Benzyl penicillin in drugs is found to contain its potassium, sodium and novocaine salts and used to be determined by the gravimetric method based on the formation of the N-ethyl piperidine salt of benzyl penicillin[3].

Phenoxymethyl penicillin is determined by UV-VIS spectrophotometry. The solvent used was a 5% sodium hydrogen carbonate solution having λ max at 268 nm. Methods of chromatographic analysis of penicillin drugs, have been developed that are employed to determine the contents of various types of penicillin present in a mixture[2].

Pipril (Piperacillin) is a relatively new bactericidal semisynthetic penicillin with a broad spectrum of activity, encompassing both ram negative and gram positive, anaerobic and aerobic organisms. The electrochemical behavior of pipril at the dropping mercury electrode is investigated by direct current polarography (DCP) and differential pulse polarography (DPP). At the hanging mercury electrode (HMDE), the reduction mechanism has been elucidated using cyclic voltametric technique in the pH range from 2 to 10. The effect of some metal ions, i.e., Cu(II) and Pd(II) has been elucidated. Detection limits of $5x10^{-8}$M in aqueous and urine samples were successfully employed[67]. Although Penicillin cannot be easily oxidized by various oxidizing agents, but the products of its alkaline hydrolysis are easily oxidized. One carbon atom is eliminated as carbon dioxide and two products are obtained in equimolecular amounts, one being amine, Penicillamine and other an aldehyde, - Penilloaldehyde. All the penicillin give the same amine, but different aldehydes[68]. The hydrolysis occurring is as : -



Hus, we considered the hydrolysis product penicillamine as the basis for the quantitative determination as its metal complex and elemental and spectroscopic characterization of its reaction product. Penicillamine is D-β,β' –dimethyl cysteine having the following structure :-



Chakrawarti et al[69-72] reported the complexation of penicillin-G, penicillin-V, carbenicillin, anexycillin and ampicillin was biologically active metal ions and found that these drugs act as bidentate ligand involving – CON- group for coordinating with the metal. The stoichiometry of these complexes is found to be 1:1 and 1:2. These complexes are in octahedral geometry. They have at least two position which are let uncoordinated and may be utilized in binding the metal penicillin complex with transptidase.

Chelation of metal ions has also been found to be important for explaining mechanisms of drug action in human body[73,74]. Chakrawati et al[75] reported the chelation of penicillin-G and penicillin-V with beryllium(II), magnesium(II) and calcium(II) using Bjerrum Calvin pH- titration technique. Be(II), Mg(II), and Ca (II) form 1:1 and 1:2 complexes with penicillin-G and penicillin-V. The proton ligand and metal ligand formation constants have calculated and the order of stability has been found to be Be(II) > Mg(II) > Ca(II). Carcinogenic metal ions possess tendency for chelation, and penicillin –G and penicillin-V form stable complexes with Fe(II), Cr(II) and Al(III), using pH –titration method. Calvin-Bjerrum's pH titration technique as adopted by Irving and Rosette was used for calculating stepwise formation constants, and the order of stability was Al(III) < Fe(III) < Cr(III). Stoichiometry of these complexes was confirmed as ML and $ML_2$.

Lead salts were found to induce cancer, specified as renal adenomas and renal adenocarcinomas. Since cancer formation and its inhibitors both involve chelation, Chakrawarti and coworkers[76] repodrted thermodynamic parameters and characterization of Pd(II). Complexes of penicillin-v and penicillin-G. Pd (II) forms 1:2 complex with penicillin-V as well as spenicillin-G Penicillamine (β-β' dimethy1 cysteine) is a prescribed drug for Wilson's disease. Rheumatoid Arthritis, and other human disease[77-80]. Wilson's disease is characterized by breakdown in copper metabolism causing excess deposition of copper in the tissues including liver and brain. The medical treatment of this rare disease includes removal of excess copper by several chelating agents like 2,3 dimercaptopropanol, EDTA, triethylene tetramine and reduced form of D-Penicillinamine[81]. Penicillamine is found to be most effective drug in curing Wilson's disease.

Colour sreaction of Penicillamine with nitroprusside and ferric chloride have been proposed[2].

Since copper is present in human body in form of different complexes, it becomes necessary to introduce a stronger chelating agent than those which are already bonded to copper. Human body plasma contains copper ions complexed in four different forms.

a. Metalloproteins are like ceruloplasmin, where copper ion is firmly bonded with inert groups of proteins.

b. Serum albumin in which copper is loosely bonded to protein.

c. Low molecular weight ligands bound to protein.

d. Low molecular weight ligands bound to the copper ions and aqua- complexes of the copper ion.

The administration of a drug like D-penicillamine librates copper from firmly and inertly complexed species like ceruloplasmin or serum albumin as low molecular wseight complexes. These low molecular weight complexes are smaller in size and preferably charged and therefore can be excreted out through kindneys. The reduced form of penicillamine is capable of increasing low molecular weight content of copper, zine and lead in plasma[82]. Which is then excreted through kindely. N-acetyl penicillamine the structural analogue of penicillamine, has been widely used for removing deleterious mercury from body. Rheumatoid arthritis is characterized by a high concentration of copper in serum. It is present as central ion in serum albumin and ceruloplsmin, which are pharmacologically inactive species. Drugs which are used for the treatment of rheumatoid arthritis include D-penicillamine, which can reduce the copper content in a manner similar to Wilson's disease and its therapy[83].

The rheumatoid arthritis is caused by the migration of activated phagocytes and leucocytes into synovial and periarticular tissuc[84]. It is the activated oxygen species mediating substances from triggered phayocytes that are responsible for enhancement of the rheumatoid condition. Excess of iron content in blood increases the arthritic inflammation owing to their pro-oxidant potentials. Drugs which are used to cure rheumatoid arthritis include gold and zinc salts. These act through a lysosomal loading sof the triggered cell, consequently reducing the toxic oxygen content of the blood. Anti rheumatic action of penicillamine involves the protection of cellular membranes against toxic oxygen by mimetic activity by the copper complex of the penicillamine for super oxidase dismutase enzyme activity. Thiols including thioimalate may act in a similar but more effective way than D-penicillamine. Observations indicate that metal compound and antioxidants can reduce anti –rheumatic action by decreasing cellular production and concentration of toxic species.

Several biological processes involve complex or chelate formation and particularly enzymes which regulate and

catalyze the important vital reactions. Biological activity of some drugs depends upon metal chelates present in the body 85,86. Complexation imparts important characteristics to the drugs, like low dissociation constant, solubility in the fluid, and their transfer through the cell membrane. When a metal complex is formed, two processes take place simultaneously.

i Transfer of electron density from the ligands (mostly organic molecules) to metal, that accelerates the bond cleavage.

ii Anactivated complex is formed between reacting molecules[87].

Penicillamine, tablet Atramin is a methylated product of aminoacid cysteine. Among the quantitative method for cysteine determination in vitro and urine samples are iodimetry[88,89]. Polarography[90] HPLC[91], ion exchange chromatography[92-95], microbilogy[96,97]. And colorimetry[98-103].

Colorimetric methods are based on the reaction with nitroprusside, phosphotungstic acid[104-110], 1-2 naphthoquinone-4 sulphonic acid[111-119] and 2,6 dichloro-p-benzoquinone[120].

Voltametric determinations have been done at concentration down to 15nm on hanging mercury drop electrode in the prsesence of nickel(II)[121(a)].

Micro amounts of penicillamine has been determined spectrophotometrically by its oxidation with Fe(III) in presence of o-Phenanthraline[121(b)].

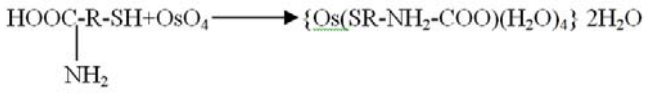
Functional groups containing nitrogen, oxygen or sulphur strong capacity to several drug molecules. The formation of a strain free five or six mebered schelating ring is favoured for action of many drugs[122].

Osmium tetraoxide scan be determined spectrophotometrically by using anthranilic acid[123] for the range 2-6 ppm of osmium.

The sensitivity is 0.0084g/cm$^2$. In the quadri, sexa and octa valent states and at s pH between 5.5 and 6.5 the reagent forms a stable dark violet 1:1 mole complex with a maximum absorbance at 460mμ with quadrivalent osmium the colour develops only upon heating for 10minutes, but with sexavalent and octavalent osmium a suffssiciently stable colour is attained in the cold after one hour. Difficulties can be experienced on the addition of the buffer if excess of Osmium are present with a deficiency of reagent. This problem is associated with methods in which colour development requires near neutral solutions. Associated platinum and base metals interfere, but EDTA serves to mask some of the interference.

O-Aminophenol-p-sulphonic acid[124] reacts with Osmium (VI) and Osmium(VIII) at pH 2.5-4 to give an intense dark brown colour with an absorbance maximum at 440mμ Maximum colour is developed in 30 minutes at room temperature. The optimum range is 2-8 ppm and sensitivity is 0.01μg/cm$^2$. Associated platinum and base metals intersfere. 1- Amino-8naphthol-3, 6- disulphonic acid[124] may be used for much the same way as 0-aminophhenol-p – sulphonic acid. The colour is developed in the pH range 4.5-6 and requires a period of 2 hours at room temperature. The sensitivity is 0.01μg/cm$^2$ and maximum absorption occurs at 480mμ 2,4- dihydroxy -3-3 nitrosquinoline (quinisation oxime) reacts with osmium (VIII) solutions to form a stable complex with a 1:2 mole ratio of metal to reagent[125]. The oxime was dissolved in a solution made up to contain 30% of N,N- dimethylformamide and 70% methanol by volume. The latter solvent was required to attain full colour development. Maximum colour was attained with at least four times the molar concentration of reagent to osmium. The reaction requires solution of pH 5 and heating to near boiling for 1.5 hours. Absorbance was measured at 515mμagainst a reagent blank. The optimum range of application is 3-10 ppm of osmium. Associated base and platinum metals interfere in proportions greater than the upper optimum range of osmium concentration. It is unfortunate that the method was not applied to caustic receiving. Solutions of octavalent oxide distillates.

During the course of systematic studies on micro determination of Os (III) by its stepwise complexation with penicillamine, it was found that it forms 1:1(M:L. ratio) complex Stoichiomatrics of 1:2 and 1:3 complexes are also observed but they are not stable due to steric hindrance of two or three penicillamine molecules. Proposed method is simple, rapid and accurate. It is useful for the determination of penicillin via its conversion to penicillamine also.




## 2. EXPERIMENTAL

### 2.1 Instruments:

Toshniwal U.V.-VIS.2000 chemito spectrophotometric observations at Bose Memorials Research Lab, Govt. Science College, Jabalpur.

Elemental analysis were carried out at RSIC, Central Drug Research Institute, Lucknow.

The FTIR spectra were recorded in Nicolet FTIR spsectrophotometer in the range 4000-400cm$^{-1}$ using KBr pellets at RSIC,IIT, Powai, Mumbai.

The ESR spectra were recorded in Varian ESR spectrometer in the scan range of 3000 gauss, Tetra cynoethyline was used as marker at RSIC. IIT Powai, Mumbai

'H NMR spectra were recorded in varian-300 MHz spectrometer using deuterium oxide as solvent at RSIC, IIT, Powai, Mumbai.

Thermal Studies (differential thermal analysis (DTA) and thermo- gravimetric analysis (TGA) were carried out in

Dupont thermal analysis system under nitrogen atmosphere from $0^0$C- $800^0$C temperature range at a rate of $20^0$C/min at RSIC. IIT, Powai, Mumbai.

**2.2 Reagents and Sample:**

Osmium Tetraoxide : A standard stock solution of Osmium tetraoxide was prepared by dissolving 1:00g of anhydrous (EMC- Electron Micros Copy Laborities LTE. Oxfsord Road, Reading, Berakshire) in up to 100ml. Solution of osmium tsetraoxide was standardized idometrically. Solutions of lower concentrations were prepared by dilution of stock solutions. Penicillin Solution: Solution of penicillin was prepared by dissolving 200 mg of procaine tablet in 100ml of double distilled water. The solution was filtered and was then hydrolysed by standardized NaOH to get penicillamine. Penicillamine thus formed is titrated iodimetrically. One mole of penicillamine reacts with three moles of standard lodine. Thus, all the reaction of penicillin is of penicillamine itself.

**Penicillamine:** Penicillamine is completely soluble in water. Standard solution of penicillamine was prepared by dissolving 250mg capsule of Artamin (U.S.P.) Biochemic Austria in 500ml of double distilled water and standardized iodimetrically **Preliminary Studies:** An aliquot of penicillamine solution ($7.331x10^{-4}$M) when mixed with equivalent amount of osmium tsetraoxide, there is no colour change. Colourless complex is formed. The complex is stable in acid mediumλ max of this complex was recorded at 238nm.

**Adherence to Beer's Law:** The complex between Penicillamine and osmium tatraoxide obeys the Beer's law in the range $1.266x10^{-1}$mg to 3.192mg, $\varepsilon = 6.70x\ 10^2$.

**Mole Ratio Studies:** Metal to ligand ratio of the complex between penicillamine and osmium (VIII) was confirmed by Job's method of continuous variance and mole ratio method, and it was found to be 1:1. The complex is stable up to 24 hours at room temperature, normal pH 6.5.

**Effect of Foreign Metal Ions:** Effect of foreign metal ions Fe(II), Fe(IV), Cu(II), Co(II), Ni(II), Pd(II) were studied and results are summarized in Table-II.

## 3. ANALYSIS

**Procedure:** The IO of spectrum is obtained by taking conductivity water in reference cell and complex solution in sample cell. And the wave length was fixed at the λ max 238nm. The change in value of absorbance was recorded and calibration curve plotted as such. The calculation for unknown amounts were performed form this calibration curve.

## 4. RESULTS AND DISCUSSION

Determination of Osmium (VIII) was achieved vdia its complexation, forming 1:1 (M:L ratio) complex spectrophotometrically, the results of determinations are summarized in Table-1. The results are accurate to < 1.12%, standard deviation and coefficient of variance $1.012x10^{-4}$, 0.0080 respectively.

While conforming the metal to lignad ratio of Osmium (VIII) and penicillamine, it was observed that there is step wise complexation of Os(VIII). The metal form 1:1 complex at first with penicillamine λ max=238nm; 1:2 and 1:3 complexes are also formed by penicillamine but they are unstable and revert back to 1:1.

Characterization of the complex of penicillamine with Os (VIII) is performed by elemental analysis, FTIR,ESR, I MR, DTA, TGA.

The percentage of carbon. Hydrogen and nitrogen for penicillamine : Os(VIII) complex is summarized in Table-III. Result are in good agreement with 1:1 M:L ratio.

**4.1 FTIR Spectrum of (Os(VIII) : Pencillamine) :**

FTIR spectra of Os (VIII): Penicillamine complex recorded in the range $400cm^{-1}$ shows the following features.

Penicillamine is β-β' dimethyl cysteine, having – SH, $-NH_2$,-COOH and $CH_3$ groups. In its FTIR spectrum broad stretching bands at 3236.6 $cm^{-1}$ and 2993 $cm^{-1}$ mays be attribsuted to symmetric and asymmetric stretch of $–NH_2$[126] whereas in Os(VIII): Penicillamine complex the band expected at $3236.6cm^{-1}$ has been found to be completely disappeared. The band at $2993cm^{-1}$ has undergone shifting to $2935.8cm^{-1}$ which indicates coordination taking place through $NH_2$ group.

A strong intense absorbance for penicillamine at $2519.2cm^{-1}$ assigned to SH group has been shifted to lower wavelength by $30.8cm^{-1}$ which is obscured due to carboxyl group. A small band at $564cm^{-1}$ is of vC-S and a band at $813.6cm^{-1}$ which is assigned to –SH bending[127] either are absent in the spectrum of the complex. This indicates the deprotonation of the sulphydryl group and coordination through sulphydryl sulphur.

A Prominent stretching band at $1354cm^{-1}$ assigned to C-N stretch[128] in the FTIR Spectrum of pure penicillamine, is almost diminished in the spectrum of its Os(VIII) complex, further confirming the involvement of amino group in complexation.

Carboxyl[129] group present in the penicillamine molecule exhibits bands at $1105cm^{1}$, $1157cm^{-1}$,$1400cm^{-1}$, $1610cm^{-1}$ cdan be assigned to stretching mode of C=O, to, bending mode of OH part of whole of the scarboxylic group[130], antisymmetric stretch mode of s-COOH, respectively, All these bands are shifted to $1021.2cm^{-1}$, $1184.2cm^{-1}$ 1469.3, 1652.6. Stretching mode of –CO is shifted to lower wavelength and symmetry stretch of COOH to higher wave number's This confirms the dimmerization and interatomic hydrogen bonding between penicillamine molecule coordinated to Os (VIII).

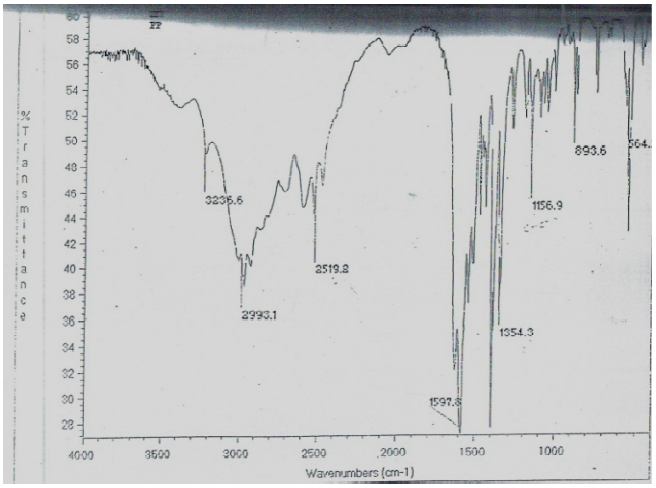



**Fig. 1(a) : FTIR SPECTRUM OF PENICILLAMINE**

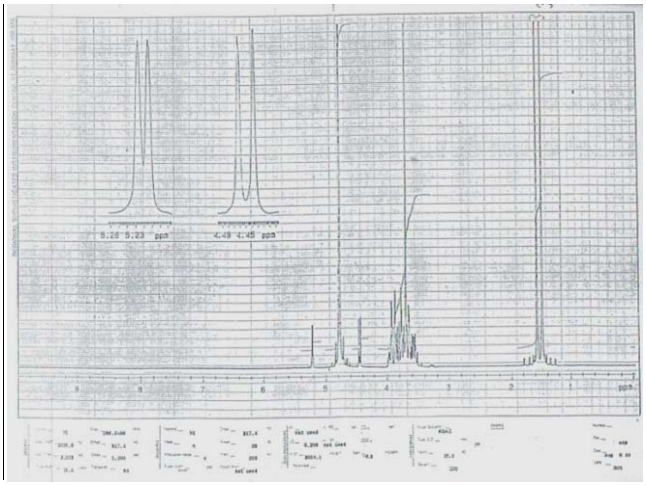


**Fig. 2 (a) :PROTON NMR SPECTRUM OF PENICILLAMINE**

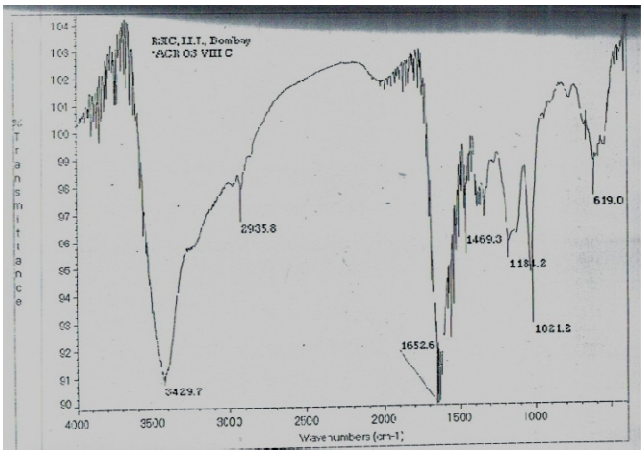



**Fig. 1 (b):FTIR SPECTRUM OF Os (VIII) : PENICILLAMINE COMPLEX**

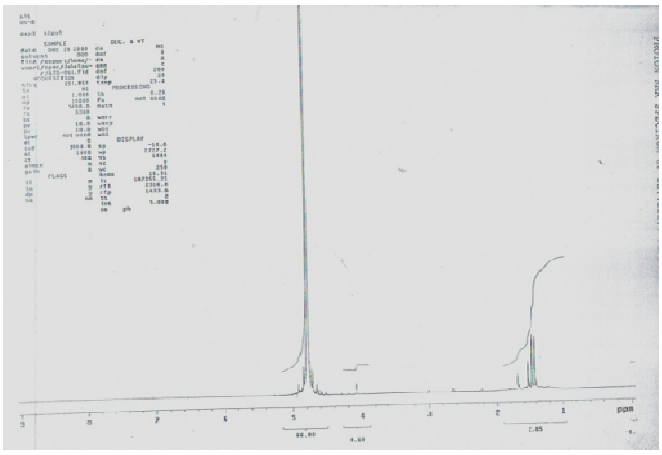


**Fig. 2 (b) : PROTON NMR SPECTRUM OF Os (VIII) : PENICILLAMINE COMPLEX**

### 4.3 $^{1}$H HMR Spectra:

The NMR spectrum of drug pencillamine and its Os (VIII) complex in $D_2O$ exhibit the following chemical shifts:

The chemical shsift at 1.2 ppm (d) in the $^{1}$H NMR spectrum of penicillamine assigned to –SH group has undergone blue shift or downfield shift to 1.46 ppm in the Os(VIII) complex confirming complexation of spenicillamine molecule via its sulphydryl group.

There is an NMR signal at 1.586 ppm as a doublet in the spsectrum of pure penicillamine attributed to the $–CH_3$ group, which has undergone upfield shift to 1.53 ppm and 1.69 ppm.

An NMR signal at 4.43 ppm in the spectrum of spenicillamine may be assigned to $–NH_2$ group. This showing 10 fold decsrease in the Os (VIII) complex establishes deprotonation of $NH_2$ group and complexation of Ox(VIII) taking place via amino group.

### 4.4 ESR Studies :

ESR specstra of pure penicillamine and Os(VIII): Penicillamime complex were recorded at room temperature exhibits following features.

No ESR signal is observed in Os(VIII): penicillamine complex indicating the diamagnetic nature of the complex.

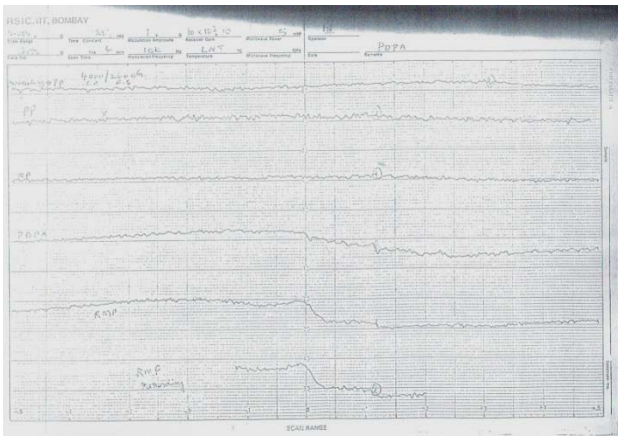


**Fig. 3 (a) :ESR SPECTRUM OF PENICILLAMINE**

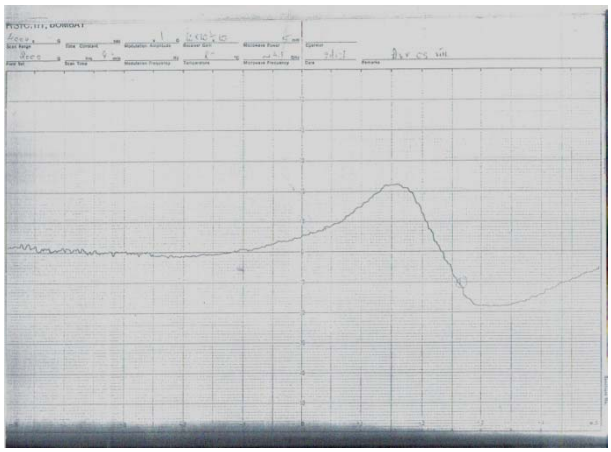


**Fig. 3 (b) : ESR SPECTRUM OF Os (VIII) : PENICILLAMINE COMPLEX**

### 4.5 Thermal Studies (TGA and DTA):

**Hermogravimetric Analysis(TGA):** The gravimetric curves of Os(VIII) : penicillamine complex were recorded out under Nitrogen atmosphere at a rate of 20"C/min from $20^0$C to $800^0$C.

The corresponding weight loss below $100^0$C is associated with the removal of lattice water molecules. Thermogram of Os(VIII): penicillamine complex shows a weigh loss of 100% to 96.05% at $66.08^0$C, which confirms the removal of two lattic water molecule from the complex.

Four sharp transitions observed in the thermogram at 152.02 $^0$C (7% weight loss). 189.52 $^0$C (5 weigh loss). 223.89 $^0$C (6% weight loss). 312.96 $^0$C (8% weight loss) are clear indication of removal of four coordinated water molecules from the Os(VIII): Penicillamine complex.

At 406.72 $^0$C (46%) weight loss is observed which is clear indication of breaking of penicillamine moiety from the complex except Os-S, remaining penicillamine decompose to give elemental carbon. Osmium in form of Os-S bond. This thermal decomposition is associated with 46% weight loss.

At 436.39 $^O$C complete breaking away of Osmium from sulphur binding site occur with a loss of 18% weight. Here too simultaneous oxidation of sulphur has been recorded.

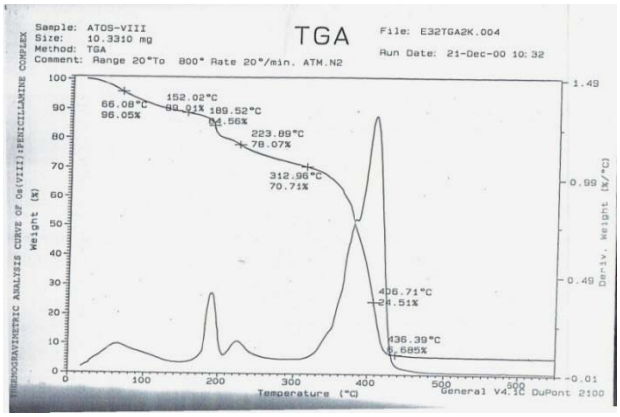



**Fig. 4: TGA SPECTRUM OF Os (VIII) : PENICILLAMINE COMPLEX**

### 4.6 Differential Thermal Analysis (DTA) :

DTA curve of the complex between Os(VIII) : penicillamine exhibits following endothermal and exothermal changes.

A small endotherm at $98^0$C is observed in DTA thermogram which is clear indication of removal of lattice water molecule.

Four exotherm from 164 $^0$C to 363 $^0$C are observed which indicate removal of coordinated water molecule from the complex. A sharp exotherm at 425 $^0$C is clear indication of breaking of drug moiety from the complex to give elemental carbon and simultaneous oxidation of sulphur to $SO_2$ takes splace.

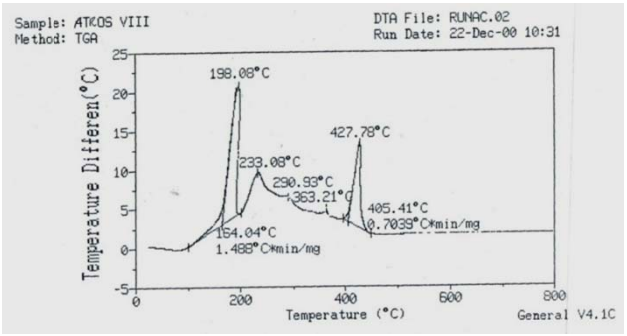



**Fig. 5: DTA SPECTRUM OF Os (VIII) : PENICILLAMINE COMPLEX**

**PROPOSED STRUCTURE OF THE COMPLEX**

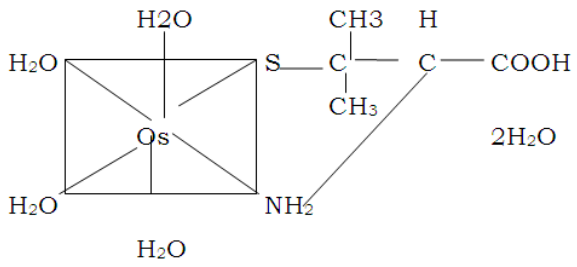



**TABLE I: MICRODETERMINATION OF THE DRUG PENICILLAMINE AS ITS Os (VIII) COMPLEX**

| S. No. | Amount of Penicillamine taken (mg) | Amount of Penicillamine found (mg) | % Error | Standard Deviation | Coefficient of Variance |
|---|---|---|---|---|---|
| 1. | $1.2600xd10^{-1}$ | $1.25x10^{-1}$ | 0.238 | $3.464x10^{-4}$ | 0.2755 |
| 2. | $1.68233x10^{-1}$ | $1.6653x10^{-1}$ | 1.010 | $1.00x10^{-3}$ | 0.6004 |
| 3. | $2.100x10^{-1}$ | $2.0958x10^{-1}$ | 0.200 | $4.85x10^{-4}$ | 0.2314 |
| 4. | $3.3600x10^{-1}$ | $3.3580x10^{-1}$ | 0.0595 | $2.309x^{-4}$ | 0.0687 |
| 5. | $3.7800x10^{-1}$ | $3.7640x10^{-1}$ | 0.4232 | $1.00x10^{-3}$ | 0.4908 |
| 6. | 1.2768 | 1.2768 | 0.00 | $3.814x10^{-4}$ | 0.0298 |
| 7. | 1.5960 | 1.5940 | 0.1254 | $2.309x10^{-3}$ | 0.1448 |
| 8. | 1.9152 | 1.9146 | 0.0313 | $6.928x10^{-4}$ | 0.0361 |
| 9. | 1.5960 | 1.5940 | 0.1254 | $2.309x10^{-3}$ | 0.1448 |
| 10. | 1.9152 | 1.9146 | 0.0313 | $6.928x10^{-4}$ | 0.0361 |
| 11. | 2.2344 | 2.2344 | 0.00 | $3.108x10^{-4}$ | 0.0139 |
| 12. | 2.5536 | 2.5528 | 0.0313 | $9.237x10^{-4}$ | 0.0362 |
| 13. | 2.8728 | 2.8726 | 0.0069 | $2.31x10^{-4}$ | 0.0080 |
| 14. | 3.192 | 3.192 | 0.00 | $1.012x10^{-4}$ | 0.0031 |

**TABLE II: EFFECT OF FORELGN METAL IONS ON Os (VIII): PENICILLAMINE COMPLEX**

| Interaferant Ion | Sample Interfernat ppm Ratio | % Recovery |
|---|---|---|
| Ferrous Ammounium Sulphate | 1:0.000056 | 100% |
| Ferric Chloride | 1:0.0372 | 99.7% |
| Copper Acetate | 1:0.002 | 99% |
| Cobalt Nitrate | 1:0.0056 | 97% |
| Nickel Sulphate | 1:0.0005 | 99% |
| Palladium Chloride | Interfere Severely | 99% |

**TABLE III: ELEMENTAL ANALYSIS OF Os(VIII): PENICILLAMINE COMPLEX (M:I. RATIO O:O)**

| Calculated | Found |
|---|---|
| % C: 17.85 | 19.88% |
| % H: 406 | 3.29% |
| % N : 4.97 | 4.16% |

## 5. ACKNOWLEDGMENT

Govt. Science College, Jabalpur. RSIC,IIT, powai, Mumbai.

## REFERENCES

[1] A. Goodman Gilman, "The Pharmacological Basis of Therapeutic", VIII Edition, Volume II, Chapter 44, Pg 1099., (1991).
[2] G. Melentyeva and L. Antonova "Pharmaceutical Chemistry"; Chapter 31, Pg. 409-433, (1988).
[3] F.S.K. Barar, "Essentials" of Pharmacotherapeutic", Chapter 9.3, P. 650-701, (1985).
[4] B.N. Sarakar, Indian J. Pharm. 24, 58, (1972)
[5] H.S.I. Tan, Pharm. Sci., 62, 993, (1973).
[6] H.G. Boixenbaum and S. Riegelman, J. Pharm. Sci. 63,1191, (1974).
[7] J.T. Stewart and D.A. Settle, J Pharm. Sci., 1403, (1975).
[8] S.R.E.L. Shabouri, S.A. Ibrahim and A.F. Youssef, Int. J. Pharm., 2,257 (1979).
[9] M.E.El- Kommos and A.S. Yanni, Analyst, 113,1091, (1988).
[10] J. Bartos, Ann. Pharm. Fr., 29,71, (1971).
[11] Y. Kidani, K. Inagoki, T. Saotome, H. Koike and Bunseki Kagaku, 22, 896, (1973).
[12] J. Vallan. A Bandinand and C. Bichon, Anal. Chim Acta., 78.93, (1975).
[13] V. J. Jennings, A Dodson and A. Harrison, Analyst, 99,145, (1977).
[14] L. Reio, J. Chromatogr., 47, 60, (1970).
[15] R.M. De SDagher, A.P. De Leenheer and A.E. Claeys, J. Chromatogr., 106, 307, (1975).
[16] A. Calo, C. Cardini and V. Quercia, Boll. Chim. Farm., 107, 296, (1968).
[17] J.T. Stewart, I.J. Honigberg, J.P. Brant, W.A. Murray, J.L. Webb and B.J. Smith, J. Pharm. Sci, 65, 1536, (1976).
[18] "British Pharmacopeia 1980", H.M. Stationery Office, London, P 244 and 780, (1980).
[19] "United States Pharmacopeia XXI, National Formulary XVT", US Pharmacopeia Convention. Rockville, Md, 566 (1985).
[20] M. Balka, D. Bode and H.J. Rhodes, J. Pharm. Sci., 63, 1303, (1974).
[21] R. Soliman and S.A. Belal, J. Durg Res., 6,7, (1974).
[22] Idem, Pharmazie, 29, 204, (1974).
[23] V.R. Nair and C.G. R. Nair, Anal. Chim. Acta., 57, 429, (1971).
[24] C.G.R. Nair and L. Kumari, Indian J. Cham., 14a, 115, (1971).
[25] M.K. Abramo, Fermatsiya. 24, 80, (1975).
[26] T. Urbanyi and R. Winchurch, J. Assoc. Off. Anal. Chem., 56, 1464, (1973).
[27] 27 L.S. Bark and P. Prachubpaibul, Anal Chim. Acta., 87, 505, (1976).
[28] G. A Brewer, Anal, Profiles sDrug Subst., 6,183, (1977).
[29] A. Alessandro, G. Meds. Mil, 104, 195, (1954); Chem, Abstr., 48, 19790d. (1954).
[30] P.V. Rao. G.B.B. Rao and P.S. Murti, Mikrochim. Acta., 979, (1974).

[31] M.T. Marrosa, Rev. Port. Farm., 19, 206, (1969); Chem, Abstr., 74, 15757d, (1971).

[32] J. Deltombe and R. Stainier, J. Pharm. Belg., 17, (1962). Chem. Abstr., 61, 5461a, (1964).

[33] O. Wollenberg, Klin. Wochschr., 30, 906, (1952). Chem. Abstr., 47, 2087i (1953).

[34] H.Kellner and H. Subhof, Klin. Wochschr., 31, 287, (1953); Chem. Abstr., 47, 7014g, (1953).

[35] R.W. Roberts and K.W. Deushle, Am. Rev. Respirat. Diseases, 80, 904, (1959); Chem. Abstr., 5510551d (1961).

[36] E. Paunescu, Acad Rep. Populare Romine, Inst. Biochim. Studii Cercetari Biochim., 4,117, (1961); Chem. Abstr., 56, 16081a, (1962).

[37] E. Paunescu and M. Buzesus, Rumanian Med. Rev., 5.38, (1961); Chem. Abstr., 57, 10502g, (1962).

[38] G.A. Smirnov and T.I. Kozulitsyna, Lab. Delo 10,48, (1964); Chem. Abstr., 60, 11039f, (1964).

[39] N. Maier and V. Moisesu, Beitr. Klin. Tuberk, 128. 123, (1964). Chem. Abstr., 61, 8773a, (1964).

[40] D. Peukert, Arzneimittel- Frosch, 7 304, (1957), Chem. Abstr., 51, 13047b. (1957).

[41] W. Wareska, Polski Tygod. Lekar., 8, 1736, (1953), Chem. Abstr., 48, 14082c, (1955).

[42] H.L. Avanza, Jornada Med. Buenos Aires, 9,673, (1955); Chem. Abstr., 50, 7003f, (1956).

[43] M.B. Jocobs,s Science, 118, 142, (1953).

[44] P. Castal, G. Carli, G. Gras. And P. Cambon, Trav. Soc Pharm. Montpellier, 15 27, (1955); Chem. Abstr., 40, 14080e. (1958).

[45] M.B. Jacob, J. Am. Pharm. Assoc., 42 346, (1953).

[46] P. Blanc, P. Bertrand and L. Lindier, Lyon Pharm. Spec., 1614. (1956); Chem. Abstr., 53, 22185c, (1959).

[47] S. Zommer and T. Lipiec, Acta Polon., 20229, (1963); Chem. Abstr., 62, 1517g. (1965).

[48] J.W. Munson and K.A. Connors, J. Pharm. Sci., 61, 211, (1972).

[49] M. Movrin, M. Jaksevac and M. Medidc –Saric, Sci. Phar., 46, 126, (1978); Chem. Abstr., 89, 18612m, (1978).

[50] M. Rizk, M.I. Walash and N.A.I. Rabeu, Arch. Pharm. Chem. Sci., Ed., 6, 26, (1978); Chem. Abstr., 89, 80270p. (1978).

[51] M.B. Devani, C.J. Shishoo, M.A. Patel and D. Bhalara, J. Pharm. Sci., 67, 661, (1978).

[52] V.V. Petrenko and L.P. Tarasyuk, Farm. Zh. (Kiev), 1,63, (1977); Chem. Abstr., 86177367b, (1977).

[53] P.R. Shah and R.R. Raje, J. Pharm, Sci., 66, 291, (1977).

[54] P.Siraj. R.R. Krishna. S.S.N. Murty, B.S. Reddy and C.S. Prakash Sastry, Talanta, 28,477-480. (1981).

[55] R.S. Satoskar and S.D. Bhandarkar, "Pharmacology and Pharmacotherapeutics", Popular Prakashan, Bombay, P. 510. (1987).

[56] V. Leon and C.sd Palmir, and R. Acad, Farm, 41(1), 159-78, (1975).

[57] V.L. Dubina and K.S. Burmistrov., Zh. Org. Khim., 13(2), 378-80, (1977).

[58] J.S. Fritz and N.M. Lisichi. Anal. Chem., 20, 589, (1951).

[59] J.S. Fritz and S.S. Yamanura, Anal Chem. 29, 1079, (1957).

[60] J.S. Friz and R.T. Keene, Anal. Chem., 24, 308- 10, (1952).

[61] P.L. De Reeder, Anal, Chem. Acta 10, 413, (1954).

[62] H.M.A. Abdini, A.M. Korany, Wahibi and F.E.I Yazpi, Talanta, 26, 1046, (1979).

[63] S. Bela, A.S. Soliman and M. Bedair, J. Durg Reg., 14(1-2), 195-201, (1983).

[64] T. Mitsui, T. Matsuoka and Y. Fujimura, Bunseki Kagaku, 34(2), 72-76, (1985).

[65] Ag. Yeuakis and M.I. Karayannis, Anal. Chem, Acta., 159, 343-347, (1984).

[66] S. Honda, T. Konishi and H. Chiba, Anal. Chem., 56(13), 2352-2354 (1984).

[67] N. Abo Ei- Maali and M.A. Ghandour, Talanta, 40, No. 12, pP. 1833-1838. (1993).

[68] I.L. Fina. "Stereochemistry and The Chemistry of Natural Products" Vol.2, V Edition, pP 865- 872, (1988).

[69] Chakrawati, P.B. Tiwari A., and Sharma H.N., (J. Ind. Chem. Soc.) Abstract, twentieth Ind. Conf, on Coord. Chem., Calcutta, India, (1979).

[70] Chakrawati. P.B., Tiwari A., and Sharma N.H., J. Chem., 19A, 83 (1979). 21 (A), 200. (1982).

[71] Chakrawati. P.B., Tiwari A., and Sharma N.H., (J.Ind. Chem, Soc.) Abstract. Twentieth Ind. J. 19a, 83 (1979). 21 (A), 200. (1982).

[72] Chakrawati. P.B., Vijay Vargiya, B.L. Shrivastava, B., Proc. Of The 57$^{th}$ Ann. Sess, Of Nat. Acad. Sci., India, No. A/70 and A/71, (1987).

[73] Coryell, C.D., Special Problems In The Formation of metal Complexes in Chemical specificity In Biological Interactions, edited by R.N. Gurd Acad. Press, New York, 90-160 (1954).

[74] Metal ions in Biological Systems, Vols 10-16, Edited by H. Sigel (mardek) N.V. 1980- 84.

[75] Chakrawati. P.B., Tiwari A., and Sharma H.N., J.IC.S.LVII, 134-6, (1980).

[76] Chakrawati. P.B., Tiwari C.P. Tiwari, A., Sharma H.N., J. I.C.S., Lxi, 705. (1984).

[77] Walshe, J.M. Am.J. Med., 21, 487, (1956).

[78] Laurie. S.H., Prime D.M., J. Inor. Biochem., 11,229, (1979).

[79] Sorensen, J.R., J. Med. Chem., 19, 135, (1976).

[80] Perrin, D.D., Agrawal, R.D., In Metal inos in Biologicdal Systems ed. H. Sigel, Marc, Dek,. New York, Vol, 2, P. 168, (1973).

[81] Fiabane, A.M. Williams, D.R., in the Principles of Bio-Inorganioc Chemistry, The Chem, Soc., No. 31p 47, (1977).

[82] Fiabane. A.M. Williams, D.R., In the Principals of Bio-Inorgainc Chemistry. The Chem. Soc., No. 31p. 57 (1977).

[83] Fiabane. A.M. Williams, D.R., In the Principals of Bio-Inorgainc Chemistry. The Chem. Soc., No. 31p. 92 (1977).

[84] Aseth, J., Hangen, M., Forre, O., Analyst, 123 (1), 3-6, (1998).

[85] Coryell, C.D., Special Problems in Formation of metal Complexes in chemical specificity Biological Interactions ed.R.N. Gurd., Acad. Pr., New York. 90-160 (1954)

[86] Metal ions sin Biological Systems, vols 10-16, ed. Sigel (Marc. Dekk) New Yorl, (1980-84)

[87] Medicinal chemistry. Ed. A. Burger (Intersciencepublishers, New York) Chapters 6-7, (1960).

[88] Y. Okuda, J. Biol Chem., 5, 638, (1925).

[89] R.W. Virtue and H.B. Lewis, J. Biol. Chem., 104, 415, (1934).

[90] G. Reed, J. Biol. Chem., 142, 61, (1942).
[91] R. Saetre and D.L. Rebenstein, Anal. Biochem., 90, 684, (1978).
[92] R.G. Westall, J. Biochem., 52, 638, (1952).
[93] W.H. Stein, J. Biol. Chem., 201, 45, (1953).
[94] R.G. Westall, J. Biochem., 60,247, (1955).
[95] D.F. Evered, J. Biochem., 62, 416. (1956).
[96] W. Frankl and M.S. Dunn. Arch. Biochem., 13, 93, (1947).
[97] M.N. Camien and M.S. Dunn, J. Boil. Chem., 183, 561, (1950)
[98] E. Brand, M.M. Harris and S. Biloon, J. Biol. Chem., 86, 315, (1930).
[99] H.B. Lewis, Ann. Intern. Med., 6, 183. (1932).
[100] C.E. Dent and G.A.Q. Rose, J. Med., 20, 205, (1951).
[101] C.E. Dent and J. M. Wolshe, "Liver Disease" Sherlock, London, P. 22. (1951)
[102] J. Fischl, I. Sason and S. Segal, Clin. Chem., 7,674, (1961)
[103] A.A. Titaev and I.I. Balabolkin, Lab. Delo., 10 618, (1971).
[104] J.M. Looney, J. Biol. Chem., 54, 171, (1922).
[105] O. Folin and A.D. Marenzi, J. Biol. Chem., 83, 103 (1929).
[106] J.W.H. Lugg, J, Biochem., 26, 2144, (1933).
[107] K. Shinohara, J. Biol. Chem., 110, 263, (1935).
[108] K. Shinohara, J. Biol. Chem., 109, 665. (1935).
[109] K. Shinohara and K.E. Padis, J. Biol. Chem., 112, 683. (1936).
[110] A. Schoberl and P. Rambacher, Z. Biochem., 295, 377, (1938).
[111] M.X. Sullivan, J. Biol. Chem. 59, 1, (1924).
[112] M.X. Sullivan, Public Health Rep., 41,1030, (1926).
[113] J.W.H. Lugg, J. Biochem., 27, 668, (1933).
[114] E. Brand. C.F. Cahill and R. Block, J. Biol. Chem., 110, 399, (1935).
[115] M.X. Sullivan and W.C. Hess, J. Biol. Chem., 116, 221, (1936).
[116] G. Medes and K.E. Padis, J. Bioshem., 30, 941, (1936).
[117] G. Medes. J. Bioshem, 30, 1293, (1936).
[118] M.X. Sullivan, W.C. Hess and H.W. Howard, J. Biol. Chem., 145, 621, (1942).
[119] Procos., J. Clin. Chem., 7,97, (1961).
[120] A.A. Femandez and R.J. Henery, Anal. Biochem., 11, 190, (1965).
[121] (A) Ana: Banica. Florinel G., Fogg. Arnold G., Kozlouwski Henry K. Electroanalysis, 8 (1), 40-3 (English) (1996).
[122] Chakrawarti, P.B., Jou. Of Sci. and Ind. Res., 48, 529- 41, (1989).
[123] A.K. Majumdar and J.G. Sen Gupta, Ibid., 20, 532, (1959).
[124] Idem, Analyt. Chem.. Acta, 22, 306, (1960).
[125] G.H. Ayres and C. Bhiggs, Analyst, Chin, Chim. Acta 26, 340, (1962)
[126] Kemp, W., Organic Spectroscopy, 3rd ed., E.L.B.S. pp. 67, (1993)
[127] Kemp, W., Organic Spectroscopy, 3rd ed., E.L.B.S. pp. 70, (1993)
[128] Kemp, W., Organic Spectroscopy, E.L.B.S. 3rd p. 67, (1993)
[129] Puthraya, K.H. and Shrivastava, T.S., Joun. of Inorg. Biochem., 26, 45-54 (1986).
[130] Silverstein, in Identification of Organic Compounds Page 101.