INTRODUCTION

The importance of penicillamine (PAM) is due to its widespread and different pharmacological effects. Penicillamine (d-3, 3 dimethyl cysteine) (Fig. 1) is used for treatment of rheumatoid arthritis, in Pb poisoning as chelating agent. The ability of D-penicillamine to act as chelating agent in therapeutic treatment of Wilson’s disease and mercury poison has been extensively investigated 1–2, and the stability constants for bivalent metals were reported and calculated from pH values using known mathematical relations and computer programming 3.

The molecule of penicillamine exist in zwitter ionic form, with three functional groups (NH2, S and COO-). This molecule usually forms bidentate complexes by coordination of N and S atoms but the possibility of forming monodentate (S) 4 tridentate (N, O, S) or tetra dentate (N, O, O, S) 5 complexes have been reported. The sulfur, nitrogen or oxygen atoms can also act as a bridge between two or more metallic atoms.

Penicillamine: Determination, Spectral and Antibacterial Studies as its Ru (III) Complex

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ABSTRACT

A spectrophotometric method has been developed for the determination of penicillamine, using Ru (III) as complexing reagent. The complex formed gives a maximum absorbance at 545 nm resulting in an orange coloured stable complex formed within 20 minutes with molar absorption coefficient 2.1x10^4 L mol^-1 cm^-1. The complex obeys Beer's law in the range of 0.099 ppm to 1.399 ppm. Stoichiometry of the complex is confirmed by Mole ratio method as well as Job’s method of continuous variance and M: L (metal: ligand) ratio was found to be 1:2.

In addition, on the basis of Elemental analysis, FTIR, H1 NMR ESR, RAMAN spectral data a plausible structure of mononuclear Ru (III) complex of penicillamine (Ru PAM2 (H2O) has been reported. Thus confirming the bonding of Ru (III) ion with sulphhydryl and amino group of penicillamine. The formed complex was further screened for the antibacterial activity against several bacteria and the results are compared with the activity of penicillamine.

Key words: Penicillamine, Spectrophotometric, Antibacterial and ruthenium (III) chloride.

INTRODUCTION

Penicillamine (d-3, 3 dimethyl cysteine) (Fig. 1) is used for treatment of rheumatoid arthritis, in Pb poisoning as chelating agent. The ability of D-penicillamine to act as chelating agent in therapeutic treatment of Wilson’s disease and mercury poison has been extensively investigated 1–2, and the stability constants for bivalent metals were reported and calculated from pH values using known mathematical relations and computer programming 3.
Penicillamine is an active ligand and easily forms complexes with transition metals. Some of its complex have been reported with mercury (II) in acidic and neutral condition with Pd (II) and Pt (II) 

\[ \text{Cu (I), Cu (II)} \] with Pb (CH₃COO)₂ and NiSO₄. However, its complexation has been exploited for reducing the nephrotoxicity with Pt antitumor drugs.

The published methods reported for the determination of penicillamine include titrimetry, spectrophotometry, fluorometry, electroanalysis and chromatography. Spectrophotometric method for the determination of penicillamine in dosage forms using 2, 6-dichloroquinone-4-chlorimide Beer's Law plot showed good correlation \((r = 0.9998)\) and the calibration graph was rectilinear over the range 4–20 mgmL⁻¹ with a detection limit of 0.15 mgmL⁻¹. The average recovery for the commercial capsules was 101.66% with an RSD of 1.57%. Colorimetric analysis of penicillamine based on Cu (II) complex formation was reported with absorption maxima of 522 nm (obtained after 30 minutes).

The use of ruthenium (III) chloride as a complexing agent for the quantitation of drugs is fairly wide. The chelate complex of ruthenium (III) ion bears the advantage of being water soluble, and hence does not necessitate any extraction procedure. Several drugs were determined spectrophotometrically by measuring the color intensity of the complex formed between their molecules and ruthenium (III) ions, e.g. ketoconazole and clotrimazole.

Some published methods suffer interference from tablets base, while others are not simple for routine analysis as they need sophisticated instruments and expensive reagents, not yet available in many control laboratories. Therefore, it was considered worthwhile to develop a rapid, simple and accurate procedure suitable for application in quality control laboratories.

The present paper reports a simple, sensitive and accurate spectrophotometric method for the determination of penicillamine. The proposed method is based on the reaction between the investigated drugs and ruthenium (III) with the formation of an orange colored complex. The optimum conditions (volume of reagent, pH etc) were established before the application of the method for the analysis of drugs as bulk or in different pharmaceutical preparations. The composition as well as the stability constant of the complex was also investigated and characterization of the complex was done by Elemental Analysis, FTIR, ¹H NMR, ESR and Raman on the basis of following equation. Other advantages of the method are cheapness and prediction of its binding sites confirming the formation of complex.

Similar complexes of Ru (III) chloride have been reported by Orioli et al. for thiopyrimidines and its derivatives 6-thiopurine riboside, and 2-thio-1, 3 pyrimidine which are investigated for their antiviral potentials as well as for photochemical properties that can be brought to design photodynamic therapies.

**EXPERIMENTAL**

**Apparatus**

An Helios δ model digital spectrophotometer provided with 1 cm quartz cells was used for absorbance measurement.

Elemental analyses were carried out in Thermo Finnigan CHNS Analyser.

The FTIR spectra were recorded in Nicolet FTIR spectrophotometer in the range 4000-400 cm⁻¹ using KBr pellets.

¹H NMR spectra were recorded in Varian 300 MHz using deuterium oxide as solvent.

ESR spectra were recorded in Varian spectrometer in the scan range of 3000 gauss using tetracyanoethylene as marker.

Laser Raman spectra were recorded by Ramanor HG 25 using Argon Laser (488nm) as a source of irradiation.

**Antibacterial activity test**

In vitro antibacterial activities of penicillamine and its complex were tested using the paper disc diffusion method. The chosen strains were G (+) Staphylococcus aureus and G (−)
Proteus mirabilis, Shigella sonnei, Salmonella enteritidis, Pseudomonas aeruginosa and Escherichia coli. The liquid medium containing the bacterial subcultures was autoclaved for 20 minutes at 121°C, at 151 lb pressure before inoculation. The bacteria were then cultured for 24 h at 36°C in an incubator. Muller-Hinton broth was used for preparing basal media for the bioassay of the organisms. Nutrient agar was poured onto a plate and allowed to solidify. The test compound (in methanol) was added dropwise to a 10 mm diameter filter paper disc placed at the centre of the agar plate. The plate is maintained at 5°C for 1 h then transferred to an incubator maintained at 36°C. The width of the growth inhibition zone around the disc was measured after 24 h incubation. Four replicas were made for each treatment.

Reagents and Chemical

All reagents used were of analytical reagent grade and the water was always double distilled obtained from Millipore.

Preparation of Penicillamine Standard Solution and its formulations

Penicillamine was kindly offered by Alembic Pharmaceutical Industry, India. The purity of the drug was determined by applying the official method 9. Stock solutions of the studied drug were prepared by dissolving 100.0 mg of the drug in 100 mL of double distilled water. Other concentrations were prepared by diluting with double distilled water.

Formulations containing 25 mg and 150 mg penicillamine were purchased from local commercial sources. Stock standard solution containing 0.005 M was prepared by dissolving the weighed amount of penicillamine in deionized water and stored in refrigerator. Standardization of the stock solution of pharmaceutical samples was done by using KIO₃ solution₃₁.

Preparation of Ruthenium (III) chloride

Hydrated ruthenium trichloride was purchased from Johnson and Matthew Co. Standard stock solution was prepared by dissolving 1g of hydrated ruthenium (III) chloride in 2 M HCl and volume is made up to 1 L. Standardization was carried out gravimetrically by dithiooxamide₃₂. Solutions of lower concentrations were prepared by approximately diluting aliquots of stock solution.

Procedure

Preparation of Complex

The complex was prepared by adding excess of penicillamine solution (7.12x10⁻⁴ M) to an equimolar solution of Ru (III) (7.49x10⁻⁴ M) taken in a flask and the contents are allowed to react for a period of 20 minutes at room temperature. The absorbance was measured against a reagent blank prepared simultaneously at the specific λₑₘ₉ (Table 1). A Beer Lambert's law graph for penicillamine and Ru (III) complex was prepared by recording and plotting the absorbance value of the concentrations of a series of penicillamine solutions of known concentration at 545 nm, which is the λₑₘ₉ of the complex.

The solid complex is prepared by evaporating the content of the complex formed. The solid sample was further purified by forming its crystallization.

Procedure for the dosage forms

An accurately weighed quantity of the mixed contents of 10 capsules equivalent to 100 mg of the drug was transferred into a 100 mL volumetric flask and the volume was made up to the mark with double distilled water. The contents of the flask were sonicated for 5 minutes and filtered if necessary and above procedure was followed for complex formation. The nominal content was calculated either from a previously plotted calibration graph or using the corresponding regression equation.

RESULTS AND DISCUSSION

Penicillamine possesses a sulphur atom, which reacts with metal ions forming stable complexes. Determination of penicillamine in the pharmaceutical drugs by complexation with a large number of metal ions such as Co²⁺, Ni²⁺ and Pb²⁺, Zn²⁺, Mn²⁺ has already been reported ³¹. All used metals for penicillamine were proved to be equally satisfactory but complexation with Ru³⁺ ion was more sensitivity, detection limit, quantification limit and A% of the formed complex was more as compared to the reported methods (Table 3).
**Table 1: Collective data of the studied compound by complexometry**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Penicillamine: RuCl₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum pH</td>
<td>Neutral</td>
</tr>
<tr>
<td>Volume of Metal solution</td>
<td>2.0 mL</td>
</tr>
<tr>
<td>Wavelength (λ max)</td>
<td>545 nm</td>
</tr>
<tr>
<td>Concentration range (µg/ml)</td>
<td>0.005 - 0.1</td>
</tr>
<tr>
<td>Y</td>
<td>2.1 x 10⁻⁴</td>
</tr>
<tr>
<td>Regression Equation Y = a + b X</td>
<td>Y = 0.01024 + 0.1379X</td>
</tr>
<tr>
<td>Slope</td>
<td>0.1379</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.01024</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9965</td>
</tr>
<tr>
<td>Molar ratio (M: L (drug))</td>
<td>1:2</td>
</tr>
<tr>
<td>Detection limit</td>
<td>0.58</td>
</tr>
</tbody>
</table>

*Y: absorbance; a: intercept; X: Concentration (µgmL⁻¹); b: slope

**Table 2: Determination of penicillamine by Ru (III) chloride**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Proposed method</th>
<th>Comparison method²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Taken (µgmL⁻¹)</td>
<td>Found (µgmL⁻¹)</td>
</tr>
<tr>
<td>Penicillamine</td>
<td>25</td>
<td>25.11</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.07</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15.16</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.88</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.97</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.09</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>100.26 ± 0.631</td>
<td></td>
</tr>
</tbody>
</table>

*Each result is the average of three separate experiments.

**Table 3: Comparison of results from the proposed method with those from other spectrophotometric methods for the determination of penicillamine in pharmaceutical formulations**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reagent used</th>
<th>λ max (nm)</th>
<th>Beer’s law limit (µgmL⁻¹)</th>
<th>RSD (%) method</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillamine</td>
<td>Ni SO₄</td>
<td>270</td>
<td>1–20</td>
<td>0.07</td>
<td>Spectrophotometric⁵²</td>
</tr>
<tr>
<td>Penicillamine</td>
<td>CoCl₂</td>
<td>291</td>
<td>2–20</td>
<td>0.14</td>
<td>Spectrophotometric⁵²</td>
</tr>
<tr>
<td>Penicillamine</td>
<td>Pb (CH₃COO)₂</td>
<td>267</td>
<td>2–25</td>
<td>0.11</td>
<td>Spectrophotometric⁵²</td>
</tr>
<tr>
<td>Penicillamine</td>
<td>2, 6-dichloroquinone-4-chlorimide (DCQ)</td>
<td>431</td>
<td>4–20</td>
<td>1.57</td>
<td>Spectrophotometric⁵²</td>
</tr>
<tr>
<td>Penicillamine</td>
<td>Ruthenium (III)</td>
<td>545</td>
<td>0.005-0.1 ppm</td>
<td>0.3-0.82</td>
<td>Present Method</td>
</tr>
</tbody>
</table>
Table 4: Determination of Penicillamine via penicillin in dosage forms by the standard addition method

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>Amount taken (mgmL⁻¹)</th>
<th>Amount added (mgmL⁻¹)</th>
<th>Total amount found (mgmL⁻¹)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pencip¹</td>
<td>25</td>
<td>25</td>
<td>49.85</td>
<td>99.71</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>150</td>
<td>300.20</td>
<td>100.10</td>
<td>0.30</td>
</tr>
<tr>
<td>Cilamin²</td>
<td>25</td>
<td>25</td>
<td>50.15</td>
<td>100.30</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>150</td>
<td>300.94</td>
<td>100.30</td>
<td>0.45</td>
</tr>
<tr>
<td>Artin³</td>
<td>25</td>
<td>25</td>
<td>49.10</td>
<td>99.40</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>150</td>
<td>300.2</td>
<td>100.10</td>
<td>0.67</td>
</tr>
</tbody>
</table>

a The results are the average of 6–separate determinations

(1) Cipla Pvt ltd, India
(2) Panacea Pvt ltd India
(3) Arvind Remedies, India

Table 5: Antibacterial activity of penicillamine: Ru (III) complex

<table>
<thead>
<tr>
<th>Name of Bacteria</th>
<th>Zone of inhibition (Penicillamine : Ru complex) (mm) Concentration</th>
<th>0.25%</th>
<th>0.5%</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>9</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Paeruginosa</td>
<td>26</td>
<td>29</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>32</td>
<td>39</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>S.sonnei</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>P.mirabilis</td>
<td>32</td>
<td>38</td>
<td>46</td>
<td></td>
</tr>
</tbody>
</table>

Estimated error ± 0.5%

Optimization of the reaction conditions

UV-Visible Spectra of Penicillamine and its Ru (III) complex

Penicillamine has maximum absorbance at 215 nm in aqueous solution, on addition of RuCl₃, two maxima are obtained one at 310 nm and other at 545 nm, whereas the latter wavelength has maximum absorbance (Figs. 4–5).

Effect of pH

The reaction between a series of penicillamine and the Ru (III) ion was performed in different media. In acid medium, low sensitivity is noticed, while in alkaline medium, the metal ions are precipitated as hydroxides. Different acidic buffers such as KCl+HCl (pH 1.6-3.5) and phosphate buffer (pH 9.5) do not show any increase in absorbance so room temperature was found to be accurate for the formation of complex.

Effect of metal ion concentration

The required amount of metal ions for maximum absorbance, besides the optimum pH, is summarized in Table 1. Absorbance was found to increase with increase in concentration of metal ion but after certain molar concentration absorbance was found to decrease.

Effect of time on the formation and stability of the formed complexes

The effect of time on the absorbance of drug metal complex was investigated. It was found that the complex formation was instantaneous and the formed complex was stable for more than 1 h.

Composition of the Complex

The M: L stoichiometric ratio of ruthenium (III) chloride: penicillamine in the complex was confirmed by Mole ratio method. The curve displayed a maximum when ruthenium concentration (7.12x10⁻⁴M) was kept constant and penicillamine concentration (7.49 x10⁻⁴ M) was varied, and the mole ratio was found to be 1:2.

Further Job’s method of continuous variance was also applied and M: L ratio was found to be 1:2.

The stability constants of the complex was calculated according to the equation:

\[ K_f = \frac{A/A_m}{(1-A/A_m)\cdot n} \cdot C^n \cdot n^n \]
where $A$ and $A_m$ are absorbance and maximum absorbance obtained from Job’s continuous variation curves; $n =$ ratio between drug to metal; $C =$ molar concentration of the drug; $K_f =$ stability formation of the complex.

The stability constant of penicillamine with the Ru (III) ion is $2.54 \times 10^4$.

Applications

By adjusting the optimum conditions required for the reaction between penicillamine with the Ru (III) metal ion, rectilinear calibration graph was obtained in the concentration range, limit of detection, regression equation and molar ratio are tabulated in Table 1. Calibration graph is shown in Fig. 3 the % recoveries of the studied drug was compared with those obtained by the former reported methods $^{11}$ are given in Tables 2, 4. Statistical analysis carried out between the results of proposed and official methods $^{11}$ are given in Tables 2 which shows no significant difference between the two procedures regarding accuracy and precision. The proposed method was successfully applied for the determination of the studied drugs in their different dosage forms, as shown Table 4, and the results obtained were compared by the reference methods $^{11,12}$. The latter methods recommend spectrophotometric method for determination of penicillamine in their dosage forms and there is no significant difference between the two procedures regarding accuracy and precision.

The complex formed as a reaction product was characterized by following spectral data.

### Characterization of the Penicillamine: Ru (III) Complex

#### Elemental Analysis

The % of carbon, hydrogen and nitrogen was determined by elemental analysis and was found to be 27.22%, 4.76%, and 4.78%, which was very much similar to the calculated value. Thus confirming the molecular structure of Penicillamine: Ru (III) complex as $(C_{10}H_{18}N_2S_2O_4Ru(H_2O)_2).Cl.2HCl$.

#### FTIR Data of the Complex

The FTIR spectrum of penicillamine (Fig. 6) and its complex have been assigned mainly for those specific frequencies, which are directly involved in complex formation. The sulphydryl group stretch band appears at 2519 cm$^{-1}$ in the spectrum of penicillamine while it is missing in the spectrum of the complex. A medium intensity band due to NH$_2$ group appears at 3236 cm$^{-1}$ is found to be shifted to 3100 cm$^{-1}$ in the spectrum of complex. All this suggests that, coordination of the ligand with metal ion occurs through the sulphur atom and nitrogen atom.

Further a small band at 564 cm$^{-1}$, corresponding to the C-S stretch is shifted to lower wave number after complexation with Ru (III) ion (Fig. 7) thus indicating coordination through the thione group. Similarly C-N stretching band at 1354 cm$^{-1}$ is shifted to 1380 cm$^{-1}$ in the spectrum of complex.

The carboxylate bands namely, (C=O) str, (-OH) bending, (COOH) antisymmetric, (-COOH) symmetric stretch arise formerly at 1050 cm$^{-1}$, 1156 cm$^{-1}$ and 1650 cm$^{-1}$.

### Scheme 1: Reaction of Penicillamine with Ru (III)

\[
\text{NH}_2
\]
\[
\begin{array}{c}
\text{H}_2\text{O} \\
2\text{CHC}(\text{CH}_3)_2 \cdot \text{SH} + \text{RuCl}_3 \\
\text{→}[\text{Ru} \cdot \text{S} \cdot (\text{CH}_3)_2 \text{CHC} \cdot \text{NHCOCH} \cdot \text{H}_2\text{O}]_2 + \text{2Cl. HCl}
\end{array}
\]

\[
\text{COOH}
\]

(Penicillamine)

(Penicillamine: RuCl$_3$ Complex)
cm⁻¹, 1400 cm⁻¹ and 1610 cm⁻¹ shows a slight shifting to 1051 cm⁻¹, 1150 cm⁻¹, 1410 cm⁻¹, 1617 cm⁻¹ in the penicillamine: Ru complex spectrum.

A broad diffuse band of medium intensity at 3414 cm⁻¹ may be assigned to the OH stretching vibration for the lattice water similar broad diffuse band has been reported by Anacona et al. Thus confirming the presence of water molecule attached to the metal ion through coordinate bond.
Fig. 4: UV-Visible spectrum of Penicillamine

Fig. 5: UV-Visible spectrum of Penicillamine: Ru (III) complex

Fig. 6: FTIR Spectrum of Penicillamine
Fig. 7: FTIR Spectrum of Penicillamine: Ru (III) complex

Fig. 8: $^1$H NMR Spectrum of Penicillamine

Fig. 9: $^1$H NMR Spectrum of Penicillamine: Ru (III) Complex
A medium intensity band at 450 cm\(^{-1}\) is due to Ru-N stretching which is quite prevalent in coordination compounds like ruthenium: xylenol complex \(^{36}\). The intensity of M-N bond also predicts the ionic character of the bond if the intensity of M—N bond and have lower stretching frequency then it is more ionic in nature \(^{37}\).

**Proton NMR Studies of Complex**

\(^1\)HNMR spectrum of penicillamine (Fig. 8) shows a peak at 1.47 ppm assigned to CH\(_3\) protons, which shows a slight shifting of 1.47 ppm peaks in complex spectrum. A sharp peak at 1.2 ppm in the spectrum of the penicillamine get reduced to half in case of complex spectrum showing the binding of sulphydryl with the metal ion (M-S) bond.

A sharp peak at 4.77 ppm is assigned to the impurity of water in D\(_2\)O, which is found in both the spectrum. As solvent used is D\(_2\)O, which involve rapid exchange of SH, and NH protons with deuterium and simplifying the spectrum thus it is...
easier to predict the presence of protons as well as confirm the binding of M-N and M-S.

A multiplet appears at 3.93 ppm in penicillamine spectrum which completely disappears and exists in the form of singlet in the spectrum of complex this is assigned to the –NH$_2$ group the reduction in the peak is mainly due to the deprotonation of primary amino group to secondary amino group while undergoing coordination with Ru (III) ion (Fig. 9).

**ESR Studies of the Complex**

Electron Spin Resonance spectrum of penicillamine (Fig. 10) and its Ru (III) (Fig. 11) complex was recorded at liquid nitrogen temperature and it exhibits the following features.

A, Calculation of g (Lande's Splitting Factor)

\[
g_{\text{Sample}} = \frac{g_{\text{Lande}} (1 - \Delta H)}{H}
\]

Where

- LH = Width between deflection points on the derivative absorption curve
- H = Magnetic field
- \(g_{\text{Lande}}\) = Lande's Factor for free electron
- \(g_{\text{Sample}}\) = 1.7231

Total Number of ESR Signals for ML$_2$ Complex.

1. Nitrogen as donor atom \(l_1 = 1\)
   \[(2 \times n \times l_1 + 1) = (2 \times 2 \times 1 + 1) = 5.\]

2. Sulphur as donor atom \(l_2 = 0\)
   \[(2 \times n \times l_2 + 1) = (2 \times 2 \times 0 + 1) = 1.\]

3. Oxygen as donor atom \(l_3 = 0\)
   \[(2 \times n \times l_3 + 1) = (2 \times 2 \times 0 + 1) = 1.\]

**Fig. 12: Laser Raman spectrum of penicillamine**

**Fig. 13: Laser Raman Spectrum of Penicillamine: Ru (III) Complex**
Total numbers of ESR Signals are.
\[(2 \times n \times I_1 + 1) \times (2 \times n \times I_2 + 1) \times (2 \times n \times I_3 + 1) = 5\]

These five resonance peaks are observed in the ESR spectrum of Ru (III): penicillamine complex confirming the presence of two penicillamine molecules in the complex.

Lower value of g obtained for the complex shows its covalent nature, owing to d⁵ electronic configuration, Ru (III) forms low spin complexes with octahedral geometry.

**Laser Raman Studies of the Complex**

Raman spectrum of penicillamine (Fig. 12) shows band at 333 cm⁻¹ assigned to -SH out of plane deformation, is absent in the spectrum of penicillamine: Ru (III) complex confirming the bonding of sulphhydryl group to Ru (III). Raman bands at 592 cm⁻¹, 665 cm⁻¹ assigned to C-SH and C-S stretch respectively are found in penicillamine spectrum which is diminishing in the complex spectrum (Fig. 13). Wagging band for – NH group appears at 752 cm⁻¹ and 853 cm⁻¹ in the spectrum of penicillamine but found to disappear in the spectrum of Ru (III): penicillamine complex. Presence of COOH in the penicillamine spectrum can also be confirmed by the bands appearing at 869 cm⁻¹. This peak becomes smaller in the spectrum of Ru (III): penicillamine complex showing the presence of uncoordinated - COOH group.

For aliphatic -CH group raman stretching frequencies are obtained at 3015 cm⁻¹ and 3024 cm⁻¹ in the penicillamine spectrum, which are shifted to 3174 cm⁻¹ and 3224 cm⁻¹ in the Ru (III) complex.

The presence of M—N bond is also confirmed by the presence of two raman active stretching modes A₁g at 500 cm⁻¹ and E_g mode at 475 cm⁻¹, generally ν A₁g is higher than ν E_g. One of the useful information obtained from these bands is that complex formed is having octahedral geometry which is the main feature of ruthenium ion.

From these studies we hereby propose the most plausible structure of Penicillamine: Ru (III) complex.

**Microbiological screening**

The susceptibility of certain strains of bacterium towards penicillamine and its metal complex was judged by measuring the size of inhibition diameter. As assessed by color, the complex remains intact during biological testing. The antibiotic and the complex presented bactericide diameter larger than 20 mm showing that they have a good activity as bactericides. The average results are shown in Table 5.

The result showed that the antibacterial activity of metal complex when compared to the parent ligand was found to increase against *Pmirabilis, E.coli* and *P aeruginosa*, whereas metal complex showed less activity against S.aureus and S.enteritidis. This phenomenon is explained on the grounds of chelation theory. Chelation reduces the polarity of the metal ion due to partial sharing of its + charge with donor groups and also due to the delocalization of electrons over the whole chelate ring. Thus Chelation increase lipophilic character in the complexes and results in enhancement of activity.

**CONCLUSION**

The proposed method is simple, accurate, precise, sensitive, very rapid, low cost and relatively selective compared to the official method. Furthermore, the proposed method does not require elaboration of procedures, which is usually associated with chromatography methods. The proposed method could be applied successfully for screening the antibacterial activity of penicillamine: ruthenium complex in different concentrations.

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