CHAPTER 5
CHARACTERISATION OF THE OXIDISED PRODUCT FOR COPPER SCHIFF BASE COMPLEXES BY AMMONIUM CERIC NITRATRE IN VARIOUS ORGANIC AND AQUEOUS SURFACTANT MICELLAR SOLUTIONS

5.1 INTRODUCTION

Protein-based radicals in enzymatic reactions are now known to function through organic side chain radicals. Several copper proteins in biology so involved in biological oxidation reaction are known to proceed through the same radical generation mechanism. Galactose oxidase catalyses the oxidation of a broad range of primary alcohols (substrate) to its corresponding aldehydes (product) through the same radical mechanism\textsuperscript{13}. The enzyme possesses a unique molecular copper site essential for catalyzing a two electron transfer reaction during the oxidation of primary alcohols to aldehydes. The interest in galactose oxidase is due to the overall reaction which is a two-electron oxidation but exists as a polypeptide chain with a single copper ion as its sole co-factor. The ability of the mononuclear active site to carry out two electron redox chemistry has been rationalized by invoking cycling between this unique Cu\textsuperscript{II} tyrosyl radical unit (active) and a Cu\textsuperscript{I} state (reduced), with the Cu\textsuperscript{II} tyrosyl form being directly responsible for hydrogen abstraction from the substrate bound in the equatorial position\textsuperscript{4-6}. 
In this chapter we report the oxidation of the copper(II) complexes of Schiff bases by Ammonium Ceric Nitrate in aqueous solutions of surfactant micelles and its catalytic activity in micellar solutions. The micellar systems simulate electrostatic and hydrophobic interactions around the active site of the complexes and helps in promoting catalysis.

5.2 EXPERIMENTAL SECTION

The synthesis of the ligand and metal complexes were explained in details in Chapter 2. The experimental procedures for the oxidation reaction of copper and zinc complexes by oxidizing agent in various solutions and solvents and their catalytic reaction are mentioned below in section 5.2(a), 5.2(b) and 5.4.

5.2(a) OXIDATION OF COPPER AND ZINC SCHIFF BASE COMPLEXES BY CERIC AMMONIUM NITRATE IN AQUEOUS SOLUTION OF SURFACTANT MICELLES

A solution of Copper and Zinc Schiff base complexes \([\text{M(Salen)}/\text{M(SalenS)}/\text{M(Salicyldehyde)}_{2}/\text{M(Sal1,3pn)}, \text{M = Cu, Zn}]\) of \(10^{-3}\)M prepared in acetate buffer of pH 4.2 was dissolved in aqueous solution of 2% CTAB prepared in acetate buffer solution of pH 4.2. \(10^{-3}\)M solution of Ammonium ceric nitrate in acetate buffer of pH 4.2 was added to the above solution. The electronic spectra of the above solutions were recorded individually and then
the spectra obtained after reaction with the oxidizing agent were recorded. The values of $\lambda_{\text{max}}$ so obtained in electronic spectra are summarized in Table 5.1.

5.2(b) OXIDATION OF COPPER AND ZINC SCHIFF BASE COMPLEXES BY CERIC AMMONIUM NITRATE IN ORGANIC SOLVENTS SUCH AS ACETONITRILE AND DIMETHYLSULPHOXIDE

A solution of Copper and Zinc Schiff base complexes $[\text{M(Salen)}/\text{M(SalenS)}/\text{M(Salicyldehyde)}/\text{M(Sal1,3pn)}, \text{M} = \text{Cu, Zn}]$ of $10^{-3}\text{M}$ is prepared in organic solvents like acetonitrile and dimethylsulphoxide. Similarly, $10^{-3}\text{M}$ solution of Ammonium ceric nitrate is prepared in acetonitrile or dimethylsulphoxide and the above solution was added to the copper solution of concentration $10^{-3}\text{M}$. The electronic spectra of the above solutions were recorded individually and then the spectra obtained after reaction with the oxidizing agent were recorded. The values $\lambda_{\text{max}}$ so obtained in electronic spectra are summarized in Table 5.2 and Table 5.3.

5.3 SPECTROSCOPIC INTERPRETATION OF OXIDATION OF Cu(Schiff base) COMPLEXES BY CERIC AMMONIUM NITRATE (CAN)

5.3(a) ELECTRONIC ABSORPTION SPECTROSCOPY
The oxidation of primary alcohols to aldehydes by Galactose Oxidase is catalysed by two-electron redox reaction at the mono-nuclear Cu\(^{2+}\) ion center \(^7\)\(^{12}\). The redox reactions of the copper protein involves the redox reaction on copper ion metal center forming Cu\(^{II}\)/Cu\(^{I}\) couple and a second redox active organic moiety in the protein which appear to be a phenoxyl radical which is in accordance with the proposed organic co-factor at the active site of the enzyme and in model systems. The catalytically active form of Copper Schiff base complexes may be written as P\(^+\)Cu(II) where P\(^+\) represents a phenoxyl radical in the Schiff base ligand \(^{13-15}\). The Cu\(^{2+}\) complex is catalytically inactive (or nearly so) unless it is activated during the reaction (probably by molecular oxygen in air). Here, we report the oxidation of Copper and Zinc Schiff base complexes by Ammonium Ceric Nitrate (CAN) and the characterization of their products by uv-visible and Electron spin Resonance spectroscopy. Oxidation of Copper Schiff base complexes by CAN at pH 4.2 (acetate buffer) in CTAB micelles gave a new band at around 444nm and that of its sulphonated derivative in aqueous micellar solutions gave a new band of 420nm. Similarly chemical oxidation of Zinc Schiff base complexes by CAN at pH 4.2 in acetate buffer solution show similar new band in the absorption spectrum at around 444nm in aqueous CTAB micellar solutions. The strong absorption bands of the oxidized complexes at around 444nm are indicative of the formation of phenoxy radical species (Fig.5.1 –
Fig. 5.3). Similar results are obtained for the Copper and Zinc Schiff base complexes in organic solvents. The electronic absorption data of the phenoxy radical species formed from the oxidation of copper complexes by CAN are reported in Table 5.1, 5.2 and 5.3.

The absorption spectra of oxidized p-cresol show a characteristic absorption at around 400nm due to phenoxy radical\textsuperscript{16-19}. The co-ordinated phenoxy radical in Cu-Duncamine complex appear at 425nm which is comparable to the one observed in Cu(Schiff base) complexes. The spectral band of the Copper and Zinc complexes in aqueous surfactant micelles is also very close to that of oxidized galactose oxidase, indicating that Copper Schiff base complexes in aqueous surfactant micelles is a very good model of galactose oxidase.

5.3(b) ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

Spectroscopic studies of Galactose Oxidase indicate that the isolated inactive enzyme contains a Cu\textsuperscript{II}-tyrosinate fragment (inactive) which upon treatment with oxidants yields the functionally competent form (active) consisting of a novel Cu\textsuperscript{II} radical pair in the active site, with the radical localized on the equatorial, covalently modified tyrosinate ligand. Supporting evidences for this hypothesized formulation includes that the catalytically active d\textsuperscript{9} Cu(II)-tyrosyl radical form is EPR- silent which is attributed to the
antiferromagnetic coupling between the two S=1/2 centers. The unpaired electron in the d_{x^2-y^2} magnetic orbital of the Cu^{2+} ion is then intramolecularly coupled to the electron in the half-occupied π orbital of the tyrosyl radical\(^{20-25}\). The Cu\(^{II}\)-phenolate radical species generated in galactose oxidase shows an intense optical absorption band at λ_{max} = 444 nm that is postulated to be due in part to a π-π* transition of the phenoxy radical.

We are able to generate Mn\(^{II}\)-phenoxy radical species that are stable in solution at low temperature by using one-electron oxidant (NH\(_4\))\(_2\)[Ce(NO\(_3\))\(_6\)] which have the added advantage of existing as spectroscopically (EPR) innocent species in their reduced forms [Ce(NO\(_3\))]\(_3\)]. Treatment of copper complex solutions in aqueous surfactant solutions in acetate buffer of pH 4.2 and organic solvents with stoichiometric amounts of (NH\(_4\))\(_2\)[Ce(NO\(_3\))\(_6\)] solution at low temperature in liquid nitrogen atmosphere resulted in a color change from violet to green. These green solutions are EPR silent (X-band, 92 ± 5% by double integration of the radical signal at 77K), consistent with magnetic coupling between an S=1/2 Cu\(^{2+}\) ion and S=1/2 phenoxy radical or antiferromagnetic coupling between a coordinated phenoxy radical and a central metal ion. The UV-Visible spectrum of the green product is similar to that of the electrochemically oxidized solution, the feature at ~415 nm \((ε \sim 3900 \text{ cm}^{-1})\) similar to that reported for the active form of Galactose Oxidase, being indicative of a coordinated phenoxy radical.
Electron Paramagnetic Resonance spectroscopy of both the copper complexes in organic as well as aqueous surfactant solution in CTAB micelles gives broadened axial spectra (Fig. 5.4 and 5.6). Copper complexes show four hyperfine splitting spectra which is consistent with tetrahedrally-distorted square planar geometry about the copper in each case.

Similarly, one-electron oxidation of Zinc Schiff base complexes was performed and the spectroscopic data for the resulting M$^{II}$-phenoxy radicals (M = Cu, Zn) are listed in Table 5.1, 5.2 and 5.3 and shown in Fig.5.1 – Fig.5.3. The UV-Visible absorption bands for the Zn$^{II}$-phenoxy radical compounds are almost similar for all zinc complexes. Finally, while both Cu$^{II}$-phenoxy radical compounds do not give rise to an EPR signal but the Zn$^{II}$-phenoxy radical compounds exhibit nearly isotropic signals (~9.07459GHz, Mod: 100 kHz; Temp: 77K, Power: 0.998W) centered at $g = 2.00463$ and 323.434mT magnetic field in their X-band EPR spectra at 77K under liquid N$_2$ atmosphere) (Fig.5.5).

5.4 CATALYTIC REACTION

The catalytic oxidation reaction of alcohols and galactose by the copper complex Cu(Sal1,3-pn) were carried as follows:

About 0.004g of Cu (Sal1,3-pn) was taken and added to 5ml of alcohol (propanol/ butanol/ benzaldehyde/ galactose) and stirred continuously in an acetate buffer solution of pH 4.2. Air was continuously passed through the
solution for 6 hours at room temperature. The aldehydic group so formed during oxidation of alcohol was identified by means of 2,4-dinitrophenylhydrazine reagent. The aldehydic compound so formed during the reaction forms 2,4-dinitrophenylhydrazone derivative with the addition of 2,4-dinitrophenylhydrazine reagent. The derivative products are further characterized by infra-red spectroscopy. The bands in the infra-red spectra were observed at 3102 cm\(^{-1}\), 1640 cm\(^{-1}\) and 1105 cm\(^{-1}\) which corresponds to \(\nu(\text{C-H})\), \(\nu(\text{C=N})\) and \(\nu(\text{N=N})\) band in the dinitrophenyldrazine derivative\(^{26,27}\).

The melting points of the product were determined and are given in Table 5.4. The melting points of the products were similar to the reported melting points of aldehydic groups as given in literature.

5.5 MECHANISM AND REACTIVITY OF COPPER COMPLEXES WITH DIOXYGEN

The copper phenolate complexes serve as structural and spectroscopic models of reduced Galactose Oxidase, providing impetus for \(\text{O}_2\) reactivity studies of these complexes with a view towards understanding how reduced Galactose Oxidase is oxidized. The copper phenolate complexes were found to be highly reactive with exposure of either solid or dissolved samples to \(\text{O}_2\) at room temperature quickly resulting in the generation of light-green species (yet to be identified)\(^{28-32}\). If the oxygenation reaction is performed at low temperature, however, intermediates can be observed, and these intermediates
have been probed by spectroscopic methods. Oxygenation of Cu(Schiff base) complexes in micellar solution as well as organic solvents at room temperature led to the generation of an EPR-silent dark green metastable intermediate with electronic absorption features at $\lambda_{\text{max}}$ 444nm. Further characterization has been hindered by the instability of this intermediate, which rapidly decays to a light-green species. Thus, we chose to focus our efforts on the characterization of more stable oxygen intermediates derived from the oxygenation of copper phenolate compounds$^{33-35}$.

The overall mechanism of the oxidation of primary alcohols to aldehydes is proposed to proceed through the formation of phenoxy radical represented as $\text{P}^+$ and $\text{P}$ is the phenolate moiety of the Schiff base ligand. It is suggested that the Cu(Schiff base) complexes in presence of alcohol is activated by molecular oxygen to a copper(II) complex containing a phenoxy radical. The radical species react with alcohol to give aldehyde and Cu$^+$ (Schiff base) complex. The Cu$^+$ complex is then oxidized by O$_2$ to the Cu$^{II}$ (Schiff base) containing the phenoxy radical.

A reasonable mechanism proposed for the two electron oxidation of alcohol to an aldehyde shows the binding of an alcohol as an alkoxide to give a five co-ordinated complex intermediate. This is followed by transfer of two electrons to [P$^+\text{Cu(II)}$] and a loss of a proton from the complexed alkoxide to
give the products. This step is similar to the one proposed for the galactose oxidase reaction.

The exact mechanism of the reaction is not yet fully understood. But the above significant experimental observation concludes that the oxidized product of Cu$^{II}$ (Salen) is likely to be due to oxidation of the ligand giving phenoxyl radical. The overall mechanism of the oxidation of primary alcohols to aldehydes is proposed to proceed through a copper(II) complex containing a phenoxyl radical. The radical species reacts with alcohol to give aldehyde and a copper(I) complex which is further oxidized by O$_2$ to the Cu$^{II}$ (Salen) containing a phenoxy radical, thus completing a catalytic cycle.

5.6 CONCLUSION

Use of modern spectroscopic and electron paramagnetic resonance techniques have shown a better progress towards the understanding of the nature of the unusual Galactose Oxidase active sites through the characterization of the Cu$^{II}$-phenolate radical species. The effect of solvent dielectric in micellar medium has shown a considerable influence on the visible absorption bands. Ligands also show a considerable influence on the uv-visible absorption bands. The model complexes are sensitive to pH and solvents.

Micellar solution of the complexes on treatment with (NH$_4$)$_2$[Ce(NO$_3$)$_6$] results in a color change. A new optical absorption band at around $\lambda_{max} =$
444nm indicates the generation of MnII-phenolate radical similar to those generated for galactose oxidase. Electrochemically generated MnII-phenolate radical species are confirmed by UV-visible spectra.

It is also demonstrated in this chapter that an aqueous surfactant solution of a copper phenolate complex in the presence of air at room temperature catalyse the oxidation of primary alcohols (such as n-propanol, n-butanol, benzyl alcohol and galactose) to their corresponding aldehydes. The Copper(I) complex is soluble and stable in aqueous micelles. In the presence of alcohol the Copper(I) complex rapidly reacts with O2 or air. From the spectroscopic and electrochemical studies it appears that the oxidized product, P+CuII(Salen), is more stable at lower pH. A reversible cyclic voltammogram of the Copper complex was obtained at pH(<5) more readily than at higher pH. Interestingly, the oxidized galactose oxidase enzyme is similarly more stable at low pH.
Fig 5.1(a) Oxidation spectra of Cu(Salen) and Zn(Salen) by Ammonium Ceric Nitrate in CTAB micelles (acetate buffer of pH 4.2).

(i) Cu(Salen)

(ii) Zn(Salen)

(iii) Overlay spectra of Cu(Salen) and Zn(Salen)
Fig 5.1(b) Oxidation spectra of Cu(Salen) and Zn(Salen) by Ammonium Ceric Nitrate in organic solvent DMSO (dimethylsulphoxide).

(i) Cu(Salen) in DMSO

(ii) Zn(Salen) in DMSO

λ_{max}(\text{nm})
444.0
Fig 5.1(c) Oxidation spectra of Cu(Salen) and Zn(Salen) by Ammonium Ceric Nitrate in organic solvent MeCN (acetonitrile)

(i) Cu(Salen)

(ii) Zn(Salen)
Fig 5.2(a) Oxidation spectra of Cu(Salicyldehyde)₂ and Zn(Salicyldehyde)₂ by Ammonium Ceric Nitrate in CTAB micelles (acetate buffer of pH 4.2).

(i) Cu(Salicyldehyde)₂

(ii) Zn(Salicyldehyde)₂

(iii) Overlay spectra of Cu(Salicyldehyde)₂ and Zn(Salicyldehyde)₂

\[ \lambda_{\text{max}}(\text{nm}) \]

\[ 444.0 \]

\[ a \quad \text{Cu(Salicyldehyde)₂} \quad 444.0 \]

\[ b \quad \text{Zn(Salicyldehyde)₂} \quad 444.0 \]
Fig 5.2(b) Oxidation spectra of Cu(Salicyldehyde)$_2$ Ammonium Ceric Nitrate in organic solvent DMSO(dimethylsulphoxide)
Fig 5.2(c) Oxidation spectra of Cu(Salicyldehyde)$_2$ by Ammonium Ceric Nitrate in organic solvent MeCN (acetonitrile)

$\lambda_{\text{max}}$(nm) = 444.0
Fig 5.3(a) Oxidation spectra of Cu(Sal1,3pn) and Zn(Sal1,3pn) by Ammonium Ceric Nitrate in aqueous surfactant solutions of CTAB micelles (acetate buffer of pH 4.2).

(i) Cu(Sal1,3pn)  
(ii) Zn(Sal1,3pn)

(iii) Overlay spectra of Cu(Sal1,3pn) and Zn(Sal1,3pn)

\[ \lambda_{\text{max}} (\text{nm}) \]

444.8 \hspace{2cm} 444.0

\[ \lambda_{\text{max}} (\text{nm}) \]

a —— Cu(Sal 1,3pn) 444.8
b —— Zn(Sal 1,3pn) 444.0
Fig 5.3(b) Oxidation spectra of Cu(Sal1,3pn) and Zn(Sal1,3pn) by Ammonium Ceric Nitrate in organic solvent DMSO (dimethylsulphoxide)

(i) Cu(Sal1,3pn)

(ii) Zn Sal1,3 pn
Fig 5.3(c) Oxidation spectra of Cu(Sal1,3pn) by Ammonium Ceric Nitrate in organic solvent MeCN (acetonitrile)

\[ \lambda_{\text{max}} (\text{nm}) \]

457.6
Fig 5.4 ESR spectra of Cu(Salen) in CTAB micellar solution in acetate buffer of pH 4.2
Fig 5.5 ESR spectra of Zn(Salen) on oxidation with Ammonium Ceric Nitrate in CTAB micellar solution in acetate buffer of pH 4.2

\[ A = 323.434\text{mT}, g = 2.00463 \]
Fig 5.6 ESR spectra of Cu(Sal1,3pn) on oxidation with Ammonium Ceric Nitrate in Acetonitrile

[A] 250.344 mT, $g = 2.58121$; [B] 263.113 mT, $g = 2.45595$; [C] 275.403 mT, $g = 2.34635$; [D] 286.099 mT, $g = 2.25863$; [E] 311.957 mT, $g = 2.07141$
Table 5.1: UV-Vis Absorption Spectral Data for $M^{II}$-Phenolate and $M^{II}$-Phenoxyl Radical Species ($M$= Cu and Zn) in acetate buffer CTAB pH 4.2

(a) Cu$^{II}$-Phenoxyl Radicals

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\lambda$ max (nm)</th>
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<tbody>
<tr>
<td>Cu(Salen)</td>
<td>444.0</td>
</tr>
<tr>
<td>Cu(Salicaldehyde)$_2$</td>
<td>444.0</td>
</tr>
<tr>
<td>Cu(Sal-1,3pn)</td>
<td>444.8</td>
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(b) Zn$^{II}$-Phenoxyl Radicals

<table>
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<th>Complex</th>
<th>$\lambda$ max (nm)</th>
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</thead>
<tbody>
<tr>
<td>Zn(Salen)</td>
<td>444.0</td>
</tr>
<tr>
<td>Zn(Salicyldehyde)$_2$</td>
<td>444.0</td>
</tr>
<tr>
<td>Zn(Sal-1,3 pn)</td>
<td>444.0</td>
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Table 5.2: UV-Vis Absorption Spectral Data for $M^{II}$- Phenolate and $M^{II}$- Phenoxy Radical Species in Acetonitrile solvent

(a) Cu$^{II}$ – Phenoxy Radicals

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<thead>
<tr>
<th>Complex</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
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<tr>
<td>Cu(Salen)</td>
<td>444.0 (sh)</td>
</tr>
<tr>
<td>Cu(Salicyldehyde)$_2$</td>
<td>444.0</td>
</tr>
<tr>
<td>Cu(Sal-1,3 pn)</td>
<td>457.6</td>
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(b) Zn$^{II}$ – Phenoxy Radicals

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<tr>
<th>Complex</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
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<tbody>
<tr>
<td>Zn(Salen)</td>
<td>444.0</td>
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</table>
Table 5.3: UV-Vis Absorption Spectral Data for $M^{II}$- Phenolate and $M^{II}$- Phenoxyl Radical Species ($M= Cu$ and $Zn$) in Dimethyl sulphoxide

(a) $Cu^{II}$ - Phenoxyl Radicals

<table>
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<th>Complex</th>
<th>$\lambda_{max}$ (nm)</th>
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<tbody>
<tr>
<td>Cu(Salen)</td>
<td>444.0 (sh)</td>
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<tr>
<td>Cu(Salicyldehydrate)$_2$</td>
<td>444.0</td>
</tr>
<tr>
<td>Cu(Sal-1,3pn)</td>
<td>444.6</td>
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(b) $Zn^{II}$ - Phenoxyl Radicals

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<th>Complex</th>
<th>$\lambda_{max}$ (nm)</th>
</tr>
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<tbody>
<tr>
<td>Zn(Salen)</td>
<td>444.0</td>
</tr>
<tr>
<td>Zn(Sal-1,3 pn)</td>
<td>444.0</td>
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Table 5.4 Melting points of the dinitrophenylhydrazone derivatives

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<thead>
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<th>Melting point (Found)</th>
<th>Melting Point (Reported)*</th>
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<tr>
<td>Propionaldehyde</td>
<td>154</td>
<td>155</td>
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<tr>
<td>Butyraldehyde</td>
<td>124</td>
<td>123</td>
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<td>Benzaldehyde</td>
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<td>237</td>
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<tr>
<td>Galactose</td>
<td>159</td>
<td>160</td>
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</table>

[* Taken from 'A handbook of organic analysis qualitative and quantitative by H.T.Clarke 5th edition, 1975, 113-115']
References


