STUDY OF NOVEL SCHIFF BASE LIGAND
WITH RESPECT TO ITS ANALYTICAL
APPLICATIONS

A PROJECT REPORT SUBMITTED TO THE
UNIVERSITY GRANTS COMMISSION

BY
DR. (MRS.) SEEMA RAJENDRA SAPALE

DEPARTMENT OF CHEMISTRY
KIRTI M. DOONGURSEE COLLEGE
DADAR, MUMBAI- 400 028
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ABSTRACT

Novel Schiff bases were synthesized from substituted 2-hydroxy benzaldehydes and dihydrazide. These Schiff bases were used to prepare metal complexes. The complexes were distinctly coloured and stable to atmospheric conditions. All newly synthesized compounds were characterized by TLC, Melting/Decomposition point, solubility, Spectroscopic Techniques (Mass, IR, NMR) and XRD (for complexes). The Schiff bases were shown to behave as a tetadentate ligand. The metal complexes were proposed to square planar geometry and 1:1 metals to ligands ratio was suggested. Some of these Schiff bases and their metal complexes were screened for their biological activities (Antibacterial and antifungal). One of the ligand was studied for spectrophotometric determination of Cu (II) (Extractive spectrophotometry). The stability constants of their complexes with Copper (II) ions and Nickel (II) ions, have been studied. Ion-selective electrode for Ni was developed.
INTRODUCTION

SCHIFF BASES

The Schiff base ligands and their metal complexes have special importance in the field of coordination chemistry. As the present work deals with the complexes of schiff base ligands, it is appropriate to include a brief general discussion on the chemistry of Schiff bases and their metal complexes.

Hugo Schiff, a German chemist, isolated the first condensed product of amines with carbonyl compounds, which he referred to as Schiff base. Thus the Schiff bases are the organic compounds containing azomethine \(-\text{R-C=O}\) group and are usually formed by the condensation of a primary amine with an active carbonyl compound. In general a Schiff base can be prepared as follows:

\[
\text{R}^{'}\text{O} \underset{\text{R'}NH}{\text{R'}} \text{R}^{''} \text{C} \text{O} + \text{H}_2\text{N-R''} \rightarrow \text{R}^{'}\text{C}\text{H}_2\text{O} + \text{R'}\text{C}\text{N-R''} + \text{H}_2\text{O}
\]

The above reaction is acid catalyzed and is generally carried out by refluxing the carbonyl compound and amine with an azeotroping agent if necessary and separating the water formed in the reaction. The kinetics and formation of Schiff bases in aqueous solution have been extensively studied. It has been found that the above reaction is \(2^{nd}\) order. This reaction proceeds by a two step mechanism involving a carbinolamine intermediate as shown below.

\[
\text{R}^{'}\text{O} \underset{\text{R'}NH}{\text{R'}} \text{R}^{''} \text{C} \text{O} + \text{H}_2\text{N-R''} \rightarrow \text{R}^{'}\text{C-NH-R''} \rightarrow \text{R}^{'}\text{C-N-R''} + \text{H}_2\text{O}
\]

Carbinolamine
This mechanism was first observed in the case of aniline and benzaldehyde in 1850 by Laurent and Gerhart. Schiff bases are also known as anils, azomethines or imines. In the polydentate Schiff base ligands, along with main functional group azomethine, the other donor sites contain various electronegative atoms such as oxygen, nitrogen and sulphur. The Schiff bases have been effectively used as analytical reagents for extractive spectrophotometric determination of metal ions, as they possess excellent ligational properties.

A considerable interest has been focused on the complexing property of bi, tri and tetradeutate Schiff base ligands having oxygen-nitrogen, oxygen-sulphur, nitrogen-sulphur and oxygen-nitrogen-sulphur as potential donor sites.

The Schiff base metal complexes have an old history, in the beginning of the 18th century preceding the work of S. M. Jorgensen and Werner, Etting in 1840 isolated a dark green crystalline product from the reaction of cupric acetate, Salicylaldehyde and aqueous ammonia. This product was undoubtedly \([\text{bis(salicylaldiimino)}\text{Cu(II)}]\). In 1869 Schiff established the 1:2 metal-ligand stoichiometry and later reported the complexes of Schiff base of urea and salicyladehyde.

![Schiff base complex](attachment:schiff_base_complex.png)

The synthesis and properties of Schiff base complexes are very often related to the associated metal and these aspects have been discussed in detail by Lindoy and Layer. Various aspects have been discussed by Mellor and Patel. Metal complexes with unusual stereochemistry and the coordination number can be
synthesized by choosing the Schiff base and carefully controlling the experimental conditions\(^\text{15}\).

Generally, Schiff bases are insoluble in water. In contrast, McCarthy et. al.\(^\text{16}\) prepared Schiff bases by condensation of ethylene diamine or triethylene-diamine with acetylacetone which found to dissociate in water. Schiff bases are weak bases and are readily hydrolyzed by dilute mineral acids, but not by aqueous alkalies.

The properties\(^\text{17}\) of the Schiff bases neither depend upon the substituent present on the nucleus nor on their position but on the characterization of \(=C=N\)- unsaturated grouping. By changing the nature and the position of the donor atoms and groups, it is possible to control the size of the chelating ring formed and exploit the effect of substitution.

The Schiff bases derived from aromatic aldehydes and primary aromatic amines are found to be more stable and show little tendency to undergo polymerization and other subsequent reactions. Schiff bases derived from lower aliphatic aldehydes and primary aromatic amines are less stable than those derived from aromatic aldehydes.

The Schiff bases are considered as useful chelating agents when a suitable functional groups like –OH, -SH, or -NO\(_2\) etc. are present sufficiently close to the azomethine group so as to form five or six membered chelating ring upon reaction with the metal ion. Most of the attention for the stability of the complexes has centered on the ligand properties and not on those of the metal ion\(^\text{18}\). For wide variety of the ligands and a series of divalent metal ions, there is a relatively invariant order of stability: Co(II) < Cu(II) < Ni(II).
Uses of Schiff bases and their metal complexes

The Schiff base ligands and their metal complexes have special importance in the field of coordination chemistry. A survey of the literature shows that, in recent times, increasing number of studies has been devoted to the synthesis and structural studies. However, the utility aspects of the Schiff bases and their metal complexes have received their share of attention with an all round progress in the field of coordination chemistry. The importance of Schiff bases and their metal complexes with transition metals have been well emphasized by several researchers.

Analytical applications

The Schiff bases have remarkable property of forming complexes and serve as excellent chelating ligands. Due to their ligational property they have been extensively used as analytical reagents19. Schiff bases formed from salicylaldehyde are used in gravimetric analysis and spectrophotometric determination. For example, the Schiff bases derived from ethylenediamine forms an insoluble complex with Ni (II), which has been used for its gravimetric determination. Also, the same reagent has been used recently for the spectrophotometric determination of Ni (II) at trace level by El-Shahawi20.

Biological Importance

The active functional group of Schiff bases is azomethine (-HC=N-) and its importance has been studied in bio-chemistry. Its biological importance has been studied in the fields of fungicides and insecticides21. Also, the intermediate of the azomethine group shows biological importance in large number of enzymatic reactions22. These compounds exhibit many physiological activities. They are found to be useful as plant growth regulants and as an anti-coagulating materials23.
AIMS AND OBJECTIVES

Objectives and the scope of the present studies:
Schiff base and its complexes have a variety of application including biological, chemical and analytical. Earlier work has shown that some drugs showed increase activity when administrated as metal chalets rather than as organic compounds. But, recently a particular attention has given to the synthesis and study of applications of Schiff bases derived from dihydrazides and substituted aldehydes and their metal complexes respectively.

General objective
- To synthesis and characterize schiff base and metal complexes derived from dihydrazide & different substituted 2- hydroxy benzaldehydes.

Specific objectives
- To characterize the schiff base type chelating agent and its complexes with some ions using spectroscopic techniques.
- To study the complexing ability and electro- analytical applications of the ligand prepared.
- To study the applications of the ligand prepared with respect to solvent extraction of some metal ions.
- To study the Biological applications of the ligand prepared and its complexes with some metal ions.
Materials and methodology
Chemicals used for this work like substituted hydroxyl benzaldehyde, hydrazine hydrate, diethyl malonate, metal salts, Ethanol, chloroform, methanol, distilled water, Acetone cobalt standard, Nickel standard etc were of analytical grade.

Apparatus and instruments
The apparatus used in this project are melting point apparatus, condenser, heating mantle, round bottom flask, rotary evaporator, beakers, measuring cylinder, ice bath, desiccators, suction pump, funnel, filter paper, stirrer etc.
EXPERIMENTAL

General procedures:

Step 1: Preparation of compound A.
Diethyl malonate (0.1mol) & Hydrazine hydrate (0.2mol) were refluxed in a 3-necked round bottom flask fitted with water condensor for 4-5 hrs. Hydrazine hydrate was added in excess. White water soluble residue precipitated out with exothermic reaction. The solid was filtered off. This is Compound A. It is recrystallized with hot absolute Alcohol (99%).

Reaction:

\[
\text{H}_2\text{N}\text{NH}_2 \xrightarrow{\text{Reflux 3-4 Hrs}} \text{NH}_2\text{NH}_2
\]

STEP 2: Preparation of compound (I). (Schiff Base Ligand) (3 Schemes)

Compound A(0.1mol) was dissolved in glacial acetic acid. Substituted 2-hydroxy-1-benzaldehyde (0.1mol) was added to it & refluxed for 3-4hrs. The reaction was monitored by TLC On cooling the reaction mixture coloured solid compound
precipitated out. The residue was filtered off and recrystallized using absolute alcohol. This is compound I. \( R = -H, -NO_2, -Br \). [I (a),(b),(c).]

**Reaction:**

\[
\text{Glacial Acetic Acid} \quad \text{Reflux} \quad 3-4 \text{ Hrs}
\]

**STEP 3: Preparation of compound (II). (Metal Complexes)**

Compound I(a) - (c) (0.1mol) was dissolved in DMF (Dimethyl formamide). Metal salt was dissolved in DMF in test tube. Both the hot solutions were mixed in a round bottom flask and refluxed for 8-10 hrs. The solution on cooling was then dumped in cold water. Colored Solid compound precipitated out. It was then filtered off & recrystallized using absolute alcohol. Here \( M = \text{Cu, Ni, Co, Cd, Zn & Pb} \). compound [I,II (a)- (f)]
Characterization of Schiff Bases & Metal complexes:

The compounds were characterized using the following parameters: TLC, Spectroscopic Techniques (Mass, IR, NMR), XRD for complexes, Melting/Decomposition point & solubility.

3.3.1 Thin layer chromatography

The completeness of the reaction was tested using thin layer chromatography precoated with silica gel plates were used for stationary phase and Chlorofrom was used as a mobile phase. The completeness of the reaction was determined by the appearance of single spot on the TLC.

3.3.2 Solubility

The Solubility of the complexes was checked by various organic solvents: diethyl ether, acetone, methanol, chloroform (CHCl3), Toluene, ethanol by stirring a small
amount of the complex in a test tubes. The ligands were found to be soluble in DMF & DMSO respectively.

3.3.3 Melting/Decomposition point)
The melting point (decomposition point) was determined by placing a finely powdered sample in a capillary tube and heating by melting point apparatus. (Model: Labtronics make, Model No: LT 110.)
CHARACTERIZATION

Various spectral, analytical and biological applications of the above Schiff bases & their complexes were studied. The details are as under:

**Spectral Analysis:**

**IR Spectra:**

The IR spectra of the Schiff bases and their metal complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000-4500 cm⁻¹ region in KBr or CsI pellets. In the IR spectra of ligands, all the absorption bands due to individual components were present except the bands at 3325-3245 cm⁻¹ due to -NH₂ in the amine and at 2750-2830 cm⁻¹ due to -HC=O group in the substituted salicylaldehyde were absent.

The spectrum of ligand exhibited weak band at 3060 per cm, this could be attributed to <(C-H) aromatic. A strong band at 1683 per cm which belongs to carbonyl and the other strong bands belong to the <(C=N), <(C=C) and <(C-N) were found at 1585, 1572 and 1244 per cm respectively, while the infrared spectra of the prepared complexes exhibited <(C =N) in the range of 1569-1577 per cm which shows a shifting to the lower frequencies, it is which indicated the coordination of (L1) with metal ions through the nitrogen atoms in their structures. The ligand(L1) shows two moderately strong bands at 3200 and 1621 per cm assigned as v O-H and v C=N groups. On complexes formation the former band shifts to lower energy while the phenolic O-H group disappears in the prepared complexes, this supports the deprotonation and linkage of O atom to the central metal ion (Erich *et al.*, 1989). The stretching frequency at 1573 per cm can be attributed to C=C bond. A strong band appearing at 1681 per cm can be assigned to carbonyl group. A medium band at 1271 per cm may be assigned to C-N stretching vibrations. The absorption band in the
range (471-535) and (423-465) per cm were assigned to (M-N) and (M-O) bands. The spectra bands of complexes at 1675-1679 per cm were characterized for the carbonyl group which did not suffer a shift. Thus, it is suggested that the oxygen atom of the carbonyl group is not coordinated to the metal ion.

**FAB-Mass Spectral Studies**

FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 kV, 10 Am) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature and m-nitro benzyl alcohol was used as the matrix. The mass spectrometer was operated in the +ve ion mode. The data thus obtained was found relevant as per projection.

The FAB mass spectrum of Schiff base (I) showed a molecular ion peak at $m/z$ 340.117 of Schiff Base (II) at $m/z$ 430.087 and that of Schiff Base (III) at $m/z$ 479.936 which is equivalent to its molecular weight. The fragments in the spectrum leads to the formation of the species $[L+H]^+$. The data is as follows:

<table>
<thead>
<tr>
<th>Schiff Base</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB 1 (I a)</td>
<td>$m/z$: 340.117 (100%), 341.121 (18.5%), 342.124 (1.6%), 341.114 (1.5%)</td>
</tr>
<tr>
<td>SB 2 (I b)</td>
<td>$m/z$: 430.087 (100%), 431.091 (18.4%), 431.084 (2.2%), 432.092 (1.6%), 432.094 (1.6%)</td>
</tr>
<tr>
<td>SB 3 (I C)</td>
<td>$m/z$: 497.936 (100%), 495.938 (51.4%), 499.934 (48.6%), 498.939 (18.4%), 496.942 (9.5%), 500.937 (9.0%), 499.943 (1.6%), 498.933 (1.5%)</td>
</tr>
</tbody>
</table>
The FAB-mass spectrum of metal complexes showed a molecular ion peak M+ equivalent to their respective molecular weights [ML₂H₂O]+. The molecular ion by the loss of two water molecules gave a fragment ion peak. All these fragments leading to the formation of the species [ML]+ which undergoes demetallation to form the species. The data is as follows:

<table>
<thead>
<tr>
<th>Metal Complexes (I)</th>
<th>M+ Ion peak Value</th>
<th>Metal Complexes (II)</th>
<th>M+ Ion peak Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I a</td>
<td>494.41</td>
<td>II a</td>
<td>528.41</td>
</tr>
<tr>
<td>I b</td>
<td>440.93</td>
<td>II b</td>
<td>474.93</td>
</tr>
<tr>
<td>I c</td>
<td>445.54</td>
<td>II c</td>
<td>479.54</td>
</tr>
<tr>
<td>I d</td>
<td>440.69</td>
<td>II d</td>
<td>474.69</td>
</tr>
<tr>
<td>I e</td>
<td>589.20</td>
<td>II e</td>
<td>623.20</td>
</tr>
<tr>
<td>I f</td>
<td>447.39</td>
<td>II f</td>
<td>481.39</td>
</tr>
</tbody>
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The ¹H NMR spectra Studies:
The ¹H NMR spectra of ligands were recorded in CDCl₃ on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. The ¹H NMR spectra of Schiff bases exhibit signals at 8.0, 5.35, 8.62 and 7.2-7.5 ppm due to -NH, phenolic OH, -CH=N and aromatic protons, respectively. The signals around 3.34 ppm are due to methylene protons. In the case of Metal (II) complex, the signal of azomethine protons of metal complexes was found at 9.12 ppm after complexations to the metal ion inferring co-ordination through the azomethine nitrogen atom of the ligands. Disappearance of -OH protons in the spectra of complexes support the deprotonation of the above group.
XRD Analysis:
The X-ray diffractograms of the Cu(II), Ni (II) complexes were scanned in the range 5–100° at a wavelength of 1.543 Å. The diffractograms and associated data depict the 2θ value for each peak, the relative intensity and inter-planar spacing (d-values). The X-ray diffraction pattern of these complexes with respect to major peaks of relative intensity greater than 10 % were indexed using a computer programme. The data shows that the ligand is tetra dented and the geometry of the complex being distorted square planar in na
APPLICATIONS

Extractive Spectrophotometry
Spectrophotometry facilitates the determination of the concentration of coloured substances in a solution and has been employed for the analysis of metals at the trace levels. Spectrophotometry is based on the quantitative study of absorption of radiation. Spectrophotometry can be classified into groups, namely UV Spectrophotometry and Visible Spectrophotometry.

UV Spectrophotometric methods have been applied extensively for the identification of aromatic hydrocarbons, vitamins, steroids, heterocyclic and conjugated aliphatic compounds. In biochemical and pharmaceutical research, UV absorption spectra are often used to identify degradation products and to test purity. The detection of characteristic UV absorption bands of contaminant is used as a guide to check the purity, while the constancy of molar absorptivity at a specific wavelength on additional purification steps is indicative of purity.

Visible Spectrophotometry involves the determination of light absorptive capacity of the chemical system, which are coloured and hence termed as colorimetric analysis or colorimetry. This method is applied to such systems when the objective is to determine the concentration of the constituent responsible for imparting colour. So one develops a coloured system and measures its absorbing capacity, which is directly proportional to the amount of desired constituent. Spectrophotometric methods are found to be simple, rapid, sensitive and can be carried out in small laboratories in the absence of the other costly and sophisticated instrumental facilities.
Solvent Extraction

Solvent extraction methods have been extensively investigated in the past few years as a promising alternative for the recovery, concentration and separation. The best evidence for the usefulness of the technique of solvent extraction in chemical science is the large number of research papers those appear in the literature, showing its analytical and industrial importance. Morrison and Freiser\textsuperscript{24} have written a comprehensive monograph on solvent extraction chemistry. The solvent extractions of metal chelate complexes have been reviewed from the stand point of chemistry by Stary\textsuperscript{25} and Zolotov\textsuperscript{26}. Mercus and Kertes\textsuperscript{27} have discussed the ion exchange and solvent extraction of metal complexes. The analytical use of solvent extraction of metals has been published by De. et al\textsuperscript{28}. Two important articles on solvent extraction of inorganic substances have been reviewed by Dimond, Tuck\textsuperscript{29} and Marcus\textsuperscript{30}. The chemical aspects, fundamentals and applications of solvent extraction have been compiled very elegantly by Sekine and Hasegawa\textsuperscript{31}.

Solvent extraction of some inorganic compounds was known in the 9\textsuperscript{th} century. The first of such examples was the extraction of uranyl nitrate into diethyl ether reported in 1842 by Peligot\textsuperscript{32}. The second worthy example was the extraction of iron from hydrochloric acid with diethyl ether reported in 1892 by Rothe\textsuperscript{33} and Hanroit\textsuperscript{34}. The method was then applied for the separation of iron from any other metal ions\textsuperscript{35}. Fischer\textsuperscript{36} introduced dithizone as a precipitant of some metal ions forming stable chelate complexes which gave widespread recognition to the solvent extraction method. Later on, extraction of many metal dithizonates was reported by Fischer\textsuperscript{37} himself. Between 1930-1950 some more chelating agents such as cupferron\textsuperscript{38}, dimethyl glyoxime\textsuperscript{39}, 1-nitroso-2-naphthol\textsuperscript{40}, 8-hydroxyquinoline\textsuperscript{41} etc. were introduced which extract metal ions as chelate complexes.
Liquid-liquid extraction commonly known as solvent extraction, is a major process step used for the separation of components of a solution by distribution between two liquid phases and is used in many process industries like petroleum refining and petrochemicals, hydrometallurgy, biotechnology and nuclear industry.

Solvent extraction has a great potential in the field of effluent treatment for economic recovery of valuable materials as well as minimizing the water pollution hazards. This method is utilized for the recovery of phenol, cresol, acetic acid and oil from the wastewater. It has been applied for inorganic metal detection such as Cu$^{2+}$ and Fe$^{3+}$ from seawater and recovery of pharmaceutically important products from wastewater. Applications of solvent extraction in wastewater treatment have been reported.

A class of organic compounds, Schiff base, also known as anil, azomethine or imine, has acquired a place of prime importance due to their varied applications when utilized as a ligand in the formation of metal complexes. In the polydentate Schiff base ligands, along with main functional group azomethine, the other donor sites contain various electronegative atoms such as oxygen, nitrogen and sulphur. The Schiff bases have been effectively used as an analytical reagent for extractive / direct spectrophotometric determination of metal ions, as they possess excellent ligational properties. Looking at the importance of the different classes of ligands, we have tried to explore the property of Schiff base ligand as an analytical reagent for extractive and / or direct spectrophotometric determination of heavy metal ions in trace levels.

The utility of solvent extraction is enhanced when it is coupled with spectrophotometric methods for separation and estimation of analyze at trace and
ultra trace level because of the associated sensitivity, selectivity, simplicity, rapidity and moderate cost per analysis. The work presented here aims at developing simple, sensitive and rapid method for extractive spectrophotometric determination of industrially and/or environmentally important heavy metals like copper (II) at trace level by using heterocyclic schiff base ligand as an analytical reagent. The method is based on instantaneous formation of stable colored complex with Cu (II) at room temperature.

The respective reagent reacts with copper (II) to give colored complex, which is extracted into organic solvent. Various experimental parameters like pH, equilibrium time, reagent concentration, stability etc. have been studied and optimized. The nature of extracted species has been determined by Job’s variation method and mole ratio method, further confirmed by the slope ratio method. The effect of foreign ions on the extraction has been studied and their tolerance limits have been determined. The proposed method has also been employed for estimation of copper in various synthetic.

Development of method

Determination of pH

To one ml of an aqueous solution containing 50 µg of copper (II), 1ml of 0.1 SB 2(Ib) in ethanol was added and the pH of the solution was adjusted to the required value by adding dilute ammonia and/or hydrochloric acid. The final volume was then made to 10 ml by using double distilled water. This solution was equilibrated with 10 ml of chloroform for 60 seconds in separating funnel. The two phases were allowed to separate, the aqueous and organic phases were collected separately and metal ion contents in both the phases i.e. amount of copper extracted into organic phase during the extraction of copper (II) with SB 2(Ib) reagent were determined.
spectrophotometrically by known method. The distribution ratio and percentage extraction values were calculated from the concentration of metal ion in each phase.

**Selection of solvents**

For selection of the suitable solvent, extraction of copper (II)- SB 2(Ib) complex was carried out at identical conditions with different solvents like toluene, carbon tetrachloride, benzene, chloroform, ethyl acetate, amyl acetate, n-butyl acetate, methyl iso-butyl ketone, iso amyl alcohol, xylene, and cyclohexanone. Distribution (D) and percentage extraction (% E) were calculated in each solvent by the procedure as mentioned above.

**Absorption spectra**

To determine the absorption maxima, 50 µg copper was used. The colored complex was extracted in selected solvent i.e. Chloroform and its absorbance was measured at different wavelength against reagent blank. The absorbance spectrum was obtained by plotting absorbance against the wavelength.

**Study of reagent concentration**

The minimum amount of reagent SB 2(Ib) required for the quantitative extraction of 50 µg metal ion was studied by varying the reagent concentration. All other parameters were kept constant. The graph of absorbance against the reagent concentration in percent was plotted. Hence, the minimum concentration of regent required for quantitative extraction of the metal ion was determined from the graph.

**Effect of equilibration time**

The effect of shaking time on the extraction of metal ion was studied. The shaking time was varied from 10 to 240 seconds the graph of absorbance verses shaking time was plotted, from which the optimum shaking time was computed.
Stability of the extracted species in organic phase

The stability of the coloured complex in organic phase was determined by measuring the absorbance of the organic phase at $\lambda_{\text{max}}$ at definite intervals of time. A graph of absorbance against the time in hours was plotted, from which the time for which the complex was stable was observed.

Calibration curve

Calibration plot was prepared by treating known amounts of copper (II) as per the method developed. Beer’s range was obtained by plotting the absorbance and the concentration of the metal ions in ppm. The calibration plot thus obtained was employed for determination of metal ion in unknown samples.

Precision and accuracy

The precision and accuracy of the developed method were determined by carrying out ten replicate analysis of solution containing known amount of metal ion. The average of these ten replicate analyses was used to calculate standard deviation, relative standard deviation and mean at 95% confidence limit.

Interference study

The effect of various cations and anions on the extraction and spectrophotometric determination of metal ion was studied at optimized experimental conditions by adding each time a definite aliquot of ion of interest to the copper solution before adjusting the pH. The tolerance limit for the foreign ion was set at the amount required to cause ± 2% error in the recovery of metal ion under investigation.
Experimental procedure for extraction and spectrophotometric determination of copper (II)

To an aliquot solution containing 0.1 to 9.0 ppm of copper (II), 1ml of 0.1 % in ethanol was added. The pH of the solution was adjusted to 9.0 by adding 2 ml of ammonium buffer and the volume was made to 10 ml using double distilled water. This aqueous phase was then taken in separating funnel and equilibrated with 10 ml of chloroform for 60 seconds. The organic extract was collected in a 10 ml flask and made upto the mark with chloroform, if necessary. The absorbance of the extracted copper (II)- SB(Ib) complex was measured at 440 nm against reagent blank. From the calibration curve, the amount of copper (II) was computed.

Procedure for spectral curve

3.934 x 10^{-4} M solutions of copper (II) and SB (Ib) were taken in different proportions and each solution mixture was treated as per the procedure mentioned earlier. The complex was extracted in 10 ml chloroform and the absorbance was recorded at different wavelengths. Thus, spectral curves were obtained by mixing the copper (II) and SB(Ib)solutions in 1:1, 1:2, 1:3, and 1:4 proportions.

Procedure for Job’s continuous variation method

A series of solutions were prepared by mixing 0.5 to 4.5 ml of 3.934 x 10^{-4} M copper (II) solution with 4.5 to 0.5 of 3.934 x 10^{-4} M SB2 (Ib) solution in such a way that the final volume was 10 ml after additions of 2.0 ml buffer of pH 9.0. Each of the molar proportion was treated as per the procedure developed and the absorbance of the extracted species was measured at 440 nm. The absorbance was plotted against the mole fraction, the ratio of concentration copper (II) to the total concentration of copper (II)- SB2(Ib).
**Procedure for Mole-ratio method**

To a series of solution containing 1.0 ml of $3.934 \times 10^{-4}$ M copper (II), increasing amounts of $3.934 \times 10^{-4}$ M SB2(Ib) volumes were added. After treating each proportion mixture as per the developed method, the absorbance of chloroform extract was measured against reagent blank at 440 nm. The absorbance values were plotted against the mole ratio of copper (II) to

**Procedure for Slope-ratio method**

To a series of solutions containing 1.0 ml of 50 µg of copper (II), varying amounts of the SB2(Ib), lesser than that the actually required for the complete extraction of copper were added. Each mixture was treated as per the developed procedure, the aqueous and organic layers were separated and concentration of copper from each layer was determined. The distribution ratio of copper (II) for each extraction was determined and a plot of log of distribution ratio Vs concentration of SB2(Ib) present in organic phase. A straight-line graph, whose, slope gives number of ligands involved in the coordination with copper (II).

Applications of extractive spectrophotometric method for determination of copper in variety of samples. The validity of proposed method was tested by applying it for the separation and determination of copper (II) in various synthetic mixtures. The developed method is found to be quite selective for the determination of copper (II).

**Procedure for determination of copper (II) in synthetic mixtures**

The method developed was employed for determination of Cu (II) in various synthetic mixtures containing other associated metal ions. Number of synthetic mixtures for Cu(II) containing other associated metal ions were prepared and
analyzed according to the procedure recommended for the respective metal ion of interest.

In a 10 ml beaker, 50 µg Cu (II) solution and known amounts of other corresponding metal ions were taken. To this mixture, 1.0 ml of 0.1% SB2(lb) solution was added and the pH of the solution was adjusted to 9.0 using buffer solution. The final aqueous volume of the mixture was made to 10.0 ml and equilibrated with 10.0 ml of chloroform for 60 seconds. The absorbance of the organic phase was determined using the calibration graph.
RESULTS AND DISCUSSION

Copper (II) Forms colored complex with BTPIMP, which was quantitatively extracted in chloroform in the alkaline medium. The extraction of Cu (II)-BTPIMP complex from the aqueous phase into organic phase was studied over wide range of experimental conditions. The results obtained during various studies are discussed below.

Effect of pH

The extraction of copper (II) was carried over the pH range of 1.0 to 11.0 [Table 5.1]. The results obtained from the pH studies indicate that the quantitative extraction (99.6 %) of copper (II) as complex occurs in the pH range of 8.0 to 10.0 [Figure 5.1]. Above and below this pH range extraction was found to be incomplete.
Solvent study

A variety of solvents were tried to achieve maximum extraction of copper (II). From the study, chloroform was found to be the most suitable solvent. The extraction of Cu (II)- SB2(Ib) complex was found to vary from a minimum of 8.57 % to maximum 92.053 % for the solvents cyclohexane < xylene < benzene < Toluene < Carbon tetrachloride < n-Butanol < Dichloromethane < Methyl isobutyl ketone < Isoamyl alcohol < Ethyl acetate < Chloroform

![Graph showing percentage extraction of copper (II) SB2(Ib) with various solvents]

Absorption spectra

The absorption spectra of the extracted species, i.e. copper (II)- complex into chloroform Vs reagent blank shows absorption maxima at 440 nm, where absorption due to reagent was found to be negligible
Reagent concentration

The effect of variation of SB2(Ib) BTPIMP in the range of 0.01 % to 0.50 % was studied for quantitative extraction of 50 µg of copper (II). 1 ml of 0.1 % was found to be sufficient concentration for the maximum extraction and spectrophotometric determination of 50 µg copper (II)

Equilibration time and stability

Extraction of copper (II) with SB2(Ib)BTPIMP as a function of equilibration time was studied for the shaking time of 10 to 240 s. The study reveals that the time of 90 seconds was found to be sufficient for quantitative extraction of copper (II) and prolong shaking had no adverse effect on extraction and spectrophotometric determination of copper (II). For further studies, the equilibration time was fixed as 90 seconds.
To study the stability of the complex, immediately after extraction, absorbance of Cu (II)- SB2(Ib)BTPIMP chloroform extract was measured against reagent blank at 440 nm and then after a definite interval of time, it was observed that the absorbance remained constant for the period of 22 hours.

Calibration curve and Sandell’s sensitivity

The plot of absorbance against concentration of copper (II) gave a straight line graph, indicating the Beer’s law is obeyed over the concentration range of 0.1 – 6.0 ppm of copper (II) at 440 nm. Optimum working range for copper (II) was found to be 1.15-7.2 ppm as evaluated by Ringbom’s plot. The Sandell’s sensitivity of the method was found to be 0.0160 µg.cm⁻² at 440 nm and the molar absorptivity was found to be 20620 lit.mol⁻¹.cm⁻¹.
**Composition of the extracted species**

The composition of the extracted species was determined by the Job’s continuous variation method and mole ratio method, which has further confirmed by using slope ratio method. Job’s continuous variation method shows a sharp maximum at 0.5 mole fraction of copper (II), indicating 1:1 stoichiometry (copper (II): SB2(Ib)). Also in mole ratio method, the plot shows a break corresponding to the mole ratio 1:1. The nature of the copper (II)- SB2(Ib) complex was further confirmed by slope ratio method, A plot of logD Vs log SB2(Ib) in the organic phase, gives a straight line with slope ≈ 1, supporting the composition of the extracted species as Cu[Sb2(Ib)].

**Precision and accuracy**

The precision and accuracy of the extractive spectrophotometric method for determination of copper (II) was calculated by analyzing the solution containing 2.5 µg of copper (II). The average for 10 determination was found to be 2.48 µg with the standard deviation 0.072, the variance 0.0052 and the variation from the mean at 95% confidence limit as 49.7 ± 0.443.

**Effect of foreign ions**

Under the optimum conditions, effect of various cations and anions on extraction of 2.5 µg of copper (II) in 10 ml solvent was studied and the tolerance limit of the diverse ion was set as the amount required to cause an error not greater than ±2 % in the percentage recovery of Cu (II). The tolerance limits for the diverse ions investigated are Br-, Cl-, F-, ClO3-, BrO3-, SO42-, NO3-, IO3-, NO2-, SO32-, S2O32-, Acetate, Citrate, Tartarate, Oxalate, thiocyanate, thiourea (20000 µg each); Na+, K+, Li+, Se4+, Ca2+, Urea, EDTA (10000 µg each); Ba2+, Al3+, V5+, (7500 µg each); S2O82-, Sb3+, Mo6+, U6+ (5000 µg each); Pt4+, W4+, Pd2+, Fe3+, Mg2+, (1500 µg each); Cr3+, Hg2+, Mn2+, Zn2+, Fe2+, Fe3+ (500 µg each); Bi3+, Sn2+, Sn4+, Mn2+, Ni2+, Ag+ (100 µg each); Cd2+, Hg2+, Co2+ (interfering).
Applications
The proposed method was applied for separation and spectrophotometric determination of copper (II) in synthetic mixtures. The recovery of Cu(II) was 99%.

Conclusion
In conclusion, the highlights of the method developed for extractive spectrophotometric determination of Cu (II) are as follows:

- The method is simple, rapid, and sensitive.
- The extraction of metal ion occurs in single step.
- For extraction of metal ions no salting out agents are required.
- The extraction of the copper metal was quantitative with the analytical reagent used without the use of any synergetic agent.
- Quantitative extraction of metal ion could be achieved within very short time.
- No pre-equilibration of phases is required; also scrubbing or back washing is not required.
- The method is relatively free from interference due to commonly associated cations and anions.
- This method has also been satisfactorily applied for determination of copper in synthetic mixtures.
Stability constant

Jannik Bjerrum developed the first general method for the determination of stability constants of metal-ammine complexes in 1941. The reasons why this occurred at such a late date, nearly 50 years after Alfred Werner had proposed the correct structures for coordination complexes, have been summarised by Beck and Nagypál. The key to Bjerrum’s method was the use of the then recently developed glass electrode and pH meter to determine the concentration of hydrogen ions in solution. Bjerrum recognised that the formation of a metal complex with a ligand was a kind of acid-base equilibrium: there is competition for the ligand, L, between the metal ion, Mn⁺, and the hydrogen ion, H⁺. This means that there are two simultaneous equilibria that have to be considered. In what follows electrical charges are omitted for the sake of generality. The two equilibria are

\[ H^+ + L \rightleftharpoons HL \]
\[ M^+ + L \rightleftharpoons ML \]

Hence by following the hydrogen ion concentration during a titration of a mixture of M and HL with base, and knowing the acid dissociation constant of HL, the stability constant for the formation of ML could be determined. Bjerrum went on to determine the stability constants for systems in which many complexes may be formed.

\[ M + qL \rightleftharpoons ML_q \]

The following twenty years saw a veritable explosion in the number of stability constants that were determined. Relationships, such as the Irving-Williams series were discovered. The calculations were done by hand using the so-called graphical methods. The mathematics underlying the methods used in this period are summarised by Rossotti and Rossotti. The next key development was the use of a
computer program, LETAGROP\textsuperscript{55,56} to do the calculations. This permitted the examination of systems too complicated to be evaluated by means of hand-calculations. Subsequently computer programs capable of handling complex equilibria in general, such as SCOGS\textsuperscript{57} and MINIQUAD\textsuperscript{58} were developed so that today the determination of stability constants has almost become a “routine” operation. Values of thousands of stability constants can be found in two commercial databases\textsuperscript{59,60}

**Experimental Study of Stability Constant:**

pH metric studies of Cu (II) & Ni (II) of substituted derivative of dihydrazide & substituted 2-hydroxy benzaldehyde.

**Procedure in Short:**

Study of the complex formation of Copper (II) ions and Nickel (II) ions with the dihydrazide & substituted 2-Hydroxy Benzaldehyde.

The stability constants of their complexes with Copper (II) ions and Nickel (II) ions, have been studied by using Irving-Rossotti method at constant temperature 27 (± 0.5)°C and ionic strength = 0.1 M. (KCl) in 75: 25 (v/v) 1, 4-dioxne-water medium. The titrations were carried out in an inert atmosphere of nitrogen.

i. 4.0 mL HCl (0.16 M) + 3.36 mL KCl (1.0 M) + 2.64 mL distilled water + 30.0 mL 1,4-dioxane.

ii. 4.0 mL HCl (0.16 M) + 3.36 mL KCl (1.0 M) + 2.64 mL distilled water + 4.0 mL ligand (L\textsuperscript{1}H) (0.04 M) + 26.0 mL 1,4-dioxane.

iii. 4.0 mL HCl (0.16 M) + requisite amount of KCl (1.0 M) solution + requisite volume of metal(II) chloride solution to give 0.001 M metal(II) chloride concentration in the final solution + requisite amount of distilled water + 4.0 mL ligand (L\textsuperscript{1}H) (0.04 M) + 26.0 mL 1,4-dioxane.
Proton ligand formation constants of the ligand and metal ligand formation constant of binary system were determined pH metrically by the method of Bjerrum and Calvin as modified by Irving and Rossotti titration technique. PH-metric titrations were carried out with systolic-μ pH meter having combined glass electronic and temperature probe maintained with readability + 0.1°C. The metal ligand ratio was maintained at 1:2. The total volume was maintained at 40 ml. All the solutions were titrated against standard carbonate free KOH (0.2 M) solution.

From the titration curves of solutions (i) and (ii), ñA values at various pH were calculated. The proton ligand formation curve was obtained by plotting the values of ñA vs. pH-meter readings. From the graph the values of log $K_{1H}$ and log $K_{2H}$ were evaluated by half Integral method (A). The values of log $K_{1H}$ and log $K_{2H}$ were also evaluated using graphical method (B) by plotting the graph of log $[ñA / (1-ñA)]$ against pH and log $[(2- ñA) / (ñA-1)]$ against pH, respectively.

**Observation:**

The dissociation constant of the Schiff bases investigated in the present work show that Schiff bases are basic in nature. The p$K_{iH}$ values are in the range of 12.0 – 12.8. The binary systems of the metal complexes were also studied. In this binary systems studied, the order of stability constants of mixed ligand complexes with respect to the metal ions was found to be Cu(II) > Ni (II) which is same as in the corresponding binary (M-L$_{sec}$) systems. This is in accordance with the Irving-Williams series of stability constant$^{61,62}$.

The values of log $K_{ter}$ are slightly lower than log $K_i$. log $K_i$ is the metal-ligand stability constant of binary complexes with corresponding secondary ligand. The relative stability of binary complexes can be qualitatively expressed in many different ways. We have expressed the relative stabilities in terms of $\Delta$ log $K_T$, $\Delta$ log $K_T = log K_{ter} - log K_i$, and log $K_i$ is the metal-ligand stability constant of binary
complexes with corresponding secondary ligand. The $\Delta \log K$ values for all the metal (II) ions in the present study is negative. This indicates that binary 1:1 (M-L$_{sec}$) complexes are stable. This behavior can be explained on the basis, that there is less number of sites available on M-L$_{pri}$ complex than on aquated metal ion.

The graphical representation of the above observations is as follows:

![Titration curves for Metal-SB2 system](image1)

![Titration curves for Metal-SB3 system](image2)
Formation curve of proton-ligand system of Schiff base (SB₂)

Half integral method   Graphical method

Formation curves for Metal (cu) -SB2 system (Half Integral Method)
Formation curves for Metal (Cu)-SB2 system (Graphical Method)

Formation curves for Metal (Ni) -SB2 system (Half Integral Method)
Formation curves for Metal (Ni)-SB2 system (Graphical Method)
ISE as an APPLICATION

Optimization of Electrode Membrane Composition

In order to prepare the electrode which has optimum response performance with respect to the specific ion under study, the effect of variation in the nature and the percentage composition of the membrane constituents on the potential response in terms of slope of the ISE was studied. Results of the optimization studies carried out for Ni(II) electrodes are given in table 3.1. The optimum membrane composition (w/w) was found to be: Resin Araldite GY 257(60.8%), Hardner HY 837(21.9%), Plasticizers dibutyl phthalate (DBP), dioctyl phthalate (DOP) or diethyl phthalate (DEP) (14.6%) and carrier / complex (2.7%). With this optimum membrane composition, the maximum and steady potential response as measured in terms of slope (mV/ decade change in concentration) for the ISE was found to be in the range 29-34mV.

Membrane preparation

The PVC-based membranes were prepared by prepared by the method of Craggs et al. [1]. Resin Araldite GY 257(60.8%), Hardner HY 837(21.9%), Plasticizers dibutyl phthalate (DBP), dioctyl phthalate (DOP) or diethyl phthalate (DEP) (14.6%) and carrier / complex (2.7%) were prepared minimum quantity of THF. The solution thus obtained, after complete dissolution of the various components, was poured into acrylic rings placed on a smooth glass plate and allowed to evaporate at room temperature. After 24h, transparent membranes of 0.5 mm thickness were obtained. A 5 mm diameter piece was cut out and glued to one end of a Pyrex glass tube. The membranes prepared above were equilibrated in 1.0 M NiCl2.7H2O were dissolved in 20 ml THF for two days and used further for potential measurement studies.
Preparation of electrode

To prepare the electrode platinum wire was coated by dipping it several times in the slurry until a bead was formed and was kept for drying in air for 24 h. Before using, the electrode was dipped in distilled water for 10 min.

Effect of Activation Time and Concentration of Activating Solution

ISEs are generally activated before use by activating or conditioning the membranes. The membranes were conditioned with Ni(II). Solutions of concentrations in the range 0.1M to 2.0M for varying length of time from 12 hours to 48 hours. It was observed that the maximum and steady response potential was obtained when the membranes were activated in 1.0M solution of respective ions for 24 hours.

Effect of Concentration of Internal Reference Solution

The optimum concentration of the internal reference solution was determined by varying the concentration in the range of 10-3M to10-1M of the respective metal ions. It was observed that the response of the electrode was better when 10-1M solution of the respective metal ion was used as internal reference solution; when the solutions of concentrations 10-2M and 10-3M were used, the response was unstable and the results were non-reproducible.

Effect of Plasticizers

The selectivities of carrier – based ISEs are highly influenced by the membrane solvent, i.e. the plasticizer. This influence is assumed to be due to the polarity of the plasticizer. This correlation is, however, only qualitative. The plasticizer also strongly influences the measuring range, i.e. the upper and the lower limits of the ISEs, through no simple correlation with its polarity alone is expected.1. It is therefore, necessary to investigate the effect of various plasticizers with a view...
to make a choice of plasticizer which gives optimum results. The effect of plasticizers on electrode performance was therefore studied. Various plasticizers like dibutyl phthalate (DBP), dioctyl phthalate (DOP) or diethyl phthalate (DEP) were used in varying proportions to examine the performance of the electrodes. It was observed that while membranes with DBP and DEP gave satisfactory performance, the results with other membranes in terms of observed slope were not encouraging. Results of membrane performance using different plasticizers are given in table 3.4 Membranes with plasticizers were found to have better elasticity in comparison to the membranes without plasticizers which were found to be brittle and gave a potential response with a lower slope per decade change in metal ion concentration. The observed slope was 30mV±2 for Nickel (II) electrode. The plasticizers DBP or DEP were, therefore selected for the use in further work.

Response Time and Stability
After optimizing the parameters like membrane composition, activation time, concentration of activating solution and concentration of internal reference solution, the response time and stability of the electrodes were also determined, as these are very important characteristics of ISEs. In earlier IUPAC recommendations, the response time was defined as the length of time between the instant at which the ISE and the reference electrode are brought in contact with a sample solution (or the time at which the concentration of the ion of interest in a solution is changed on contact with an ISE and a reference electrode) and the first instant at which the potential of the cell becomes equal to its steady state value within 1mV2 or has reached 90% of the final value3. More recently, it has been extended to be able to treat the drifting systems as well. In this case, the second time instant is defined as the time at which the slope, E/t, becomes equal to limiting value 4,5. The response time was determined by dipping the electrodes into the solutions of respective metal ion of concentration 10-5M to 10-1M and the potential response was recorded at
short intervals of 30 seconds each. The maximum time required to obtain a steady potential response was found to be 60 seconds. The study of variation of the electrode potential with the length of time of immersion of the electrode in solution showed a stable and reproducible potential. The life-span or stability of an ion selective electrode with respect to time is an important factor in the use of any electrode. The life-spans of the electrodes were determined by measuring the response potential of the electrode at different time intervals. The electrodes were found to be effective over a period of two months if stored properly by dipping in 0.01M solution of the respective metal ion.

Effect of pH
The pH dependence of the response potential Ni(II) ISEs was investigated over the pH range of 2 to 12 for 10-2M and 10-4M solutions of the respective ions. It was observed that the potential response remains constant in the working pH range 3.0-6.0 for Ni (II) electrode and Ni (II) electrode containing dibutyl phthalate and diethyl phthalate as plasticizers. Above and below these pH ranges, a sharp change in potential response was observed. The pH dependence of the potential response can be expected as the complex formation between the metal ions and the complexing ligands in the membrane matrix proceeds through the replacement of a proton by the corresponding metal ion.

Conclusion
The proposed ion selective membrane electrodes for Nickel is easy to prepare and can be used for the determination of Ni (II) ions in solution in the range 10-1M to 10-5 M. The functional pH range lies between 3.0 - 6.0 for Ni (II) electrode. Various experimental parameters have been optimized. The response time for all the electrode is about 02 minutes and the electrodes are stable for about two weeks, if stored properly. The presence of several other ions tested does not
significantly interfere with the sensitivity of the ISE. The electrodes have been successfully employed for the determination of the respective ions in samples including chemicals and sea water. The results obtained compare well with those obtained by alternative methods.

**Biological activity**

Some of the metal complexes of the above synthesized Schiff bases were tested for their antibacterial & antifungal activities only.

**Procedure in short:** The biological activities of synthesized Schiff base and its Cu (II), Ni (II) metal complexes were studied for their antibacterial and antifungal activities. Following bacterial and antifungal culture which were used for the experiments.

**Bacterial Cultures**

i) Escherchia Coli ATCC 8739 (Gram negative rod)
ii) Bacillus Subtilis ATCC6633 (Gram positive rod)
iii) Staphylococcus Aureus ATCC6538 (Gram positive cocci)
iv) Streptococcus Epidermidis ATCC12228 (Gram Positive cocci)
v) Nircococus Luteus ATCC 10240 (Gram Positive cocci)
vi) Psudomonas Aerogenosa ATCC 9027 (Gram Positive rods)

**Fungal Cultures**

i) Candida albocans ATCC 10231
ii) Aspergilus niger ATCC 16404

Above bacterial/fugal cultures were plated in agar plate and were incubated at respective conditions with test solutions filled in the hole punctured in the agar plate
(for bacterial culture optimum conditions is 25°C for 24 hrs for Bacteria and 48°C for fungus)

**Observation**

The distance between the edge of the hole and the edge of the inhibitory zone is measured and used in evaluation. No zone of inhibition was observed. These compounds don’t have any antibacterial or antifungal effect but may have other properties like antiviral, anticancer etc which needs to be tested.
REFERENCES


47. J. P. Clark, Food Tech., 58(7), 2004, 78.


