CHAPTER – IV
HETEROCYCLIC AZO DYES IN SPECTROPHOTOMETRIC ANALYSIS

4.1 INTRODUCTION

Azo dyes have attracted much attention as they are sensitive chromogenic reagents in addition to interesting complexing nature and have been used as reagents for spectrophotometric and extractive photometric determinations of many metal ions. Some of them are also proved to be particularly useful as indicators in complexometric titrations owing to the low stabilities of the metal indicator complexes.

In the subsequent pages of this Chapter, an attempt has been made to outline a survey of relevant literature regarding the preparation, purification, mechanism, general complexing properties of azodyes and applications of the heterocyclic azo dyes derived from 2-amino pyridines, 2-aminothiazoles and 2-aminobenzothiazoles as complexometric indicators and spectrophotometric reagents.

4.2 AZO DYES

Azo dyes are largest group of organic reagents used in spectrophotometric analysis. They are also found in a variety of industrial applications mainly because of their colour fastness and low price. These compounds have the general formula R-N=N-R', in which R and R' can be either alkyl or aryl. The -N=N- group is called an -azo group, although the parent compound, H-N=N-H is called di-imide. The more stable derivatives contain two aryl groups. Azo dyes are used for colouring numerous consumer goods, such as leather, clothes, food, toys, plastics and cosmetics [Ahlstrom L. et al., 2005].
4.2.1 Preparation and Purification of Azo Dyes

A primary amine (R-NH₂) is converted into a diazonium salt and this is reacted with another aryl unit to yield azo dye.

![Diagram of the preparation of an azo dye](image)

Usually dyes are purified by repeated crystallizations from ethanol. Dissolution of the dye in alkali metal hydroxide solution, followed by extraction with diethyl ether and subsequent re-precipitation with dilute hydrochloric acid, has proved to be useful for purification of thiazole dyes. Some azo dyes are purified by sublimation under reduced pressure. Drying the dye over calcium chloride in desiccators, or drying the dye at 120°C removes the water content. The azo dyes can be separated from impurities such as phenol and other azo dye derivatives by paper chromatography, thin layer chromatography and column chromatography [Havard R. Hovind, 1975].

4.2.2 Mechanism of Azo Dye Formation

Step 1: Diazotation

When primary aryl amines react with nitrous acid (HNO₂, generated from NaNO₂ and HCl), a reaction occurs which makes a diazonium ion. The reaction takes place under 0-5°C conditions.

![Diagram of the Diazotation reaction](image)
Step 2 – Coupling reaction

The diazotized sulphanilic acid reacts with 2-naphthol and 1-naphthylamine to produce an acidic and basic azo dye respectively. The azo coupling reaction represents an electrophilic aromatic substitution. The diazonium cation is a relatively weak electrophile. So, the aromatic ring which attacks must have an activating group such as -OH, -NH₂. Electron withdrawing groups on the aromatic rings of the diazonium ion facilitate the substitution reaction. Electrophilic substitution of 2-naphthol occurs preferentially at the 1-position. The -NH₂ group in 1-naphthylamine activates the 2- and 4-positions.

In azo compounds the -N=N- group is part of an extended delocalized electron system involving the aromatic rings, called - chromophore. Groups such as -OH and -NH₂ attached to chromophores modify the colours of the dyes.

4.2.3 General properties of azo dyes

Generally azo dyes are red, violet or brownish in their crystalline state and few are readily soluble in water, most of them being only partly soluble or insoluble. However, their solubility can be increased by the addition of organic solvents, e.g., chloroform, cyclohexane and butan-1-ol, than in water.
4.2.4 Complexing properties of azo dyes

Dyes give coloured complexes with most of the metals; stable chelates being, especially with some of the transition metals. In acidic and slightly acidic solutions, the metals form complexes with a metal to ligand ratio of 1:1 or mixture of complexes 1:1 and 1:2 in alkaline solutions, the equilibrium usually displaces towards the 1:2 complex [Venkatraman K., 1970].

Among the various available azo dyes, heterocyclic azo dyes are well known for their outstanding photometric sensitivities, constitute an important class amongst the various organic reagents used in the determination of metal ions [Anderson R.G. et al., 1967; Shibata S. et al., 1972; Sandell E.B. et al., 1978; Chauhan O.S. et al., 1980; Qiufen Hu et al., 2004; Garg Bhagwan et al., 2005]. Heterocyclic azo compounds belong to aromatic azo compounds, but they make up an individual group of organic reagents, both by the peculiarities of their synthesis and by their reactivity and analytical characteristics. The credit for synthesizing of heterocyclic azo compounds belongs to Chichibabin A.E. et al. (1915). They obtained 2-aminopyridine and 2-aminoquinoline and comprehensively studied diazotization of 2-aminopyridine and it's azo coupling, leading to the synthesis of heterocyclic azo compounds. At present, the numbers of heterocyclic azo compounds are used in analytical chemistry substantially. It totals several hundred compounds and some of them are of special interest as analytical reagents. In addition to 2-aminopyridine, 2-amino derivatives of pyrazole, imidazole, antipyrine, triazole, thiazole, benzothiazole, quinoline and other heterocycles (more than 15) are used.

Heterocyclic azo compounds interact with all elements existing in solutions as cations. Alkali metals are the exceptions. It is reported that 1-(2-pyridyl azo)-2-naphthol interacts with alkaline earth elements to form extractable complexes [Ivanov V.M., 2005].

Heterocyclic azo dyes of pyridyl azo, thiazolyl azo and benzothiazolyl azo dyes are important class of organic reagents with good complexing capacity. The complexes are formed through the coordination bonds with the donor
Nitrogen atom of the heterocyclic system, Nitrogen atom of the azo group and the hydroxy group at the ortho position to the azo group.

\[
\text{N} = \text{N} \quad \text{HO}
\]

**Heterocyclic azo compounds containing analytical functional group.**

The application of these azo dyes in spectrophotometry is based on the colour resulting from their reaction with most of the metals, especially with some transition metals. Usually, stable chelates are produced. Generally, these dyes have been employed in separation procedures. The reagents and their metal complexes have rather limited solubility in aqueous solutions but much greater solubility in organic systems. They are also employed as indicators.

### 4.3 HETEROCYCLIC AZO DYES AS COMPLEXOMETRIC INDICATORS

The most common application is the direct titration of the metal against EDTA solution with the dye as indicator, the metal being displaced from its dye complex at the end point. The blue, green or violet colour of the solution due to the complex changes to that of the free ligand (yellow or orange). The rate of the colour change of the indicator near the equivalence point is governed by the rate of substitution reaction between the metal indicator complex and the titrant at room temperature, which is often slow, but its rate can be increased by heating the solution to 60-70°C near the end point. Few examples of heterocyclic azo dyes used in the complexometric titration are given in Table 4.1 [Chromy V. and Sommer L., 1967; Anderson R.G. and Nickless, 1967; Kai F., Izumi H., 1970].
Table 4.1: Heterocyclic azo dyes as complexometric indicators

<table>
<thead>
<tr>
<th>Name of the indicators</th>
<th>Metal ions</th>
<th>Specific characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-(2-pyridyl azo)-2-naphthol (PAN)</td>
<td>Bi(III), Pb (II), Ga(III), In(III),Ti(III),Zn(II),Cd(II), Hg(II), Cu(II), Ni(II), Co(II), Fe(II), Mn(II), Al(III), Mg(II), Ca(II), Sc(III), Ce(III), Th(IV)</td>
<td>PAN is more useful in indirect and replacement titration than in direct titration, in which its most important application is the titration of Copper. PAN suffers from the disadvantage of being, like its chelates, insoluble in water and many solutions require heating during titration with PAN as indicator.</td>
</tr>
<tr>
<td>4-(2-Pyridyl azo)-resorcinol (PAR)</td>
<td>Bi(III),Pb(II),Ga(III),In(III), Ti(III), Zn(II),Cd(II),Hg(II), Cu(II), Ni(II), Mn(II),Al(III)</td>
<td>PAR is an alternative for PAN in complexometric titrations. Immediate advantage of PAR over PAN is that this reagent and most of its complexes are water soluble.</td>
</tr>
<tr>
<td>4-(2-pyridyl azo)-orcinol and 1-(2-pyridyl azo)-2,3-dihydroxy naphthalene-6-sulphonic acid (PADNS)</td>
<td>Metals similar to those of PAN</td>
<td>PADNS produce red colour with metal ions.</td>
</tr>
<tr>
<td>7-(2-pyridyl azo)-8-hydroxy quinoline, (PAOx)</td>
<td>Ti(III) and Cu (II)</td>
<td>PAOx Chelates at a lower pH than either PAN or PAR.</td>
</tr>
<tr>
<td>2-(2-pyridyl azo)-2-naphthol (β-PAN)</td>
<td>Cu (II)</td>
<td>As an extractive indicator in titration of EDTA with Copper.</td>
</tr>
<tr>
<td>2-(2-pyridyl azo)-4-methylphenol (PAC)</td>
<td>Cu (II), Zn (II), Cd(II), Pb (II)</td>
<td>Better indicator than PAN and PAR giving sharp colour change from violet to yellow at the end point.</td>
</tr>
<tr>
<td>4-(2-pyridyl azo)-phenol</td>
<td></td>
<td>Acid base indicator in non-aqueous media.</td>
</tr>
<tr>
<td>1-(2-thiazolyl azo)-2-naphthol (TAN)</td>
<td>Co (II), Cu(II), Zn(II)</td>
<td>Indicators, in both direct and indirect titrations.</td>
</tr>
<tr>
<td>2-(2-thiazolyl azo) chromotropic acid(Tach)</td>
<td>Zr(IV), Th(IV)</td>
<td>Indicators, in both direct and indirect titrations.</td>
</tr>
<tr>
<td>Thiazolylazo dyes of resorcinol, orcinol, p-cresol, m-dimethyl amino phenol, 2-naphthol, and 2-naphthol sulphonic acid</td>
<td>Co (II), Cu (II), Ni(II)</td>
<td>Most useful indicators for direct titrations. Addition of ethanol and heating were required for the sharpest end point.</td>
</tr>
</tbody>
</table>
4.4 PYRIDYL AZO, THIAZOLYL AZO AND BENZOTHIAZOLYL AZO DYES IN THE SPECTROPHOTOMETRIC ANALYSIS

4.4.1 Pyridyl azo dyes as Spectrophotometric reagents

2-amino pyridine can be diazotized under the action of isoamyl nitrite in anhydrous ethanol in the presence of sodium alcoholate (or sodium amide). The stable diazotate is coupled with any phenolic compound yields pyridyl azo dyes.

1-(2-Pyridyl azo)-2-naphthol (PAN) and most of its chelates are insoluble in water, so they are extracted in organic solvents. After chelation, the metal passes from aqueous to organic layer. Chloroform is the usual choice, but isopentyl alcohol [Cheng K.L. and Bray R.H., 1955], benzene [Ho Li-Shu et al., 1965], o-dichlorobenzene [Cheng K.L., 1958], ether and carbon tetrachloride [Shibata S., 1963] have also been suggested.

\[
\text{HO} \quad \text{N} \quad \text{N} \\
\text{1-(2-Pyridyl azo)-2-naphthol}
\]

PAN was used for the spectrophotometric determination of various metal ions like Ga(III), In(III), Ti(I), Ti(III), Zn(II), Cd(II), Hg(II), Cu(II), Ag(I), Ni(II), Pd(II), Co(II), Rh(II), Ir(III), Fe(III), Mn(II), Zr(IV), Y(III), U(VI), Ca(II) and Pb(II). Absorbance varies from 540 to 615 nm depending upon the metal ions. Almost all the methods of metal determinations using PAN include extraction procedure.

4-(2-Pyridyl azo)-resorcinol (PAR) and its complexes are water-soluble. Spectrophotometric analysis with PAR includes mixed solvent systems. The sensitivity of PAR for metal ions is greater than that of PAN. It was reported to be most sensitive reagent for Cobalt and also most sensitive water-soluble reagent for uranium, being 30% more sensitive than PAN.
Except in the determination of palladium, all optical densities are measured at wavelengths of 494 to 550 nm. With PAN, a wide range of wavelengths was used, which gives a great stability of choosing a part of the spectrum, in which the interference of foreign ions is at minimum. This increases the selectivity of PAN and makes possible certain simultaneous determinations. Such methods are not possible with PAR. Also selective solvent extraction is not applicable, which shows that PAR is less selective reagent than PAN. PAR is used for the determination of metal ions like Pb(II), Sn(II), Ga(III), In(III), Ti(III), Cu(II), Pd (II), Co(II), Fe(III), Os(IV), V(V), Nb(V), Ta(V), Ti(IV), Th(IV), La(III), Ce(III), U(VI), Np(V). Absorbance varies from 490 to 550 nm depending on the metal ions. Spectrophotometric determination of minute amounts of Cu(II) by extracting its PAR complex in the presence of tetradecyl dimethyl benzyl ammonium chloride with chloroform at pH 9.7 and absorbance at 510 nm was reported and the method was recommended for water analysis [Nonova D. et al., 1982].

2-(2-Pyridyl azo)-4-methylphenol (PAC) act as bidentate ligand in the spectrophotometric determination of Cu(II), Ni(II) and Zn(II) [Nakagawa et al., 1962].

5-(2-Pyridyl azo)-2-monoethyl amino-p-cresol (PAAC) was used for the spectrophotometric determination of Thallium and Cobalt, 2-(2-pyridyl azo)-2-
naphthol (β-PAN) for spectrophotometric determination of Indium. [Gusev S.I. et al., 1964,1966]

2-[2-(5-Bromo pyridyl) azo]-5-dimethyl aminophenol (5-Br-DMPAP) was used as a sensitive reagent for the spectrophotometric determination of Cadmium by solvent extraction with 3-methyl-1-butanol. 1:2 complex formed showed the stability at pH 9 and \( \lambda_{\text{max}} \) 555nm [Shibata S. et al., 1976].

2-(3,5-dibromo-2-pyridyl azo)-5-diethyl aminophenol (3,5-di Br-PADAP) was applied to the determination of silver (I) in the presence of anionic surfactant [Shui-Chieh Hung et al., 1982].

![4-(3,5-dibromo-2-pyridylazo)-5-diethyl amino phenol](image)

The water-soluble bidentate ligand, 4-(3,5-dibromo-2-pyridyl azo)-N-ethyl-N-(3-sulpho propyl) aniline (3,5-di Br-PAESA), provides greatest sensitivity, forming a (1:2) Copper:Ligand in presence of sodium dodecyl sulphate with maximum absorption of the complex at 638 nm. The method was applied for the determination of Copper (II) in serum [Ohshita K. et al., 1985].

![4-(3,5-dibromo-2-pyridyl azo)-N-ethyl-N- (3-sulpho propyl) aniline](image)
Among all the pyridyl azo compounds, 1-(2-pyridyl azo)-2-naphthol (PAN), 4-(2-pyridyl azo) resorcinol (PAR) and 2-(5-bromo-2-pyridyl azo)-5-diethyl amino phenol (5 Br-PADAP) are still most widely used. Chemical industries of many countries manufacture these reagents.

4.4.2 Thiazol and Benzothiazolyl azo dyes as Spectrophotometric reagents

2-amino thiazole and 2-amino benzothiazoles are like their pyridyl analogues, which require no special conditions for diazotization and hence thiazolyl azo dyes and benzothiazolyl azo dyes are easily prepared from a wide range of phenols, naphthols and the other non-phenolic substances.

Diazotisation reaction occurs readily in concentrated solutions of hydrochloric, phosphoric and sulphuric acids or in a mixture of dimethyl formamide and sulphuric acid. The diazonium salt formed is so reactive that, it has to be coupled with the phenol in acidic solution, which often results in the immediate production of the dye in crystalline state.

2-(2′-Thiazolylazo)-5-dimethyl aminophenol (TAM) was sensitive reagent for the spectrophotometric determination of uranium [Sorensen E., 1960].

Much important work on thiazolyl azo compounds was carried out in Japan. Initially Yanagihara and Kawase prepared 2-(4-methyl-2-thiazolyl azo)-4-methoxy phenol, which was used for the spectrophotometric determination of Copper, Zinc, Nickel, Cobalt and Iron [Yanagihara T., Matano N. and Kawase A., 1958]. 2-(2-thiazolyl azo) phenol and their 23 derivatives were synthesized and used them for the spectrophotometric determination of Ni(II), Co(II) and Fe(III). Among the dyes studied 2-(2-thiazolyl azo) naphthol (TAN) was the most useful for the spectrophotometric determination of the Zinc [Yanagihara T., Matano N.
et al., 1959; Matano N. et al., 1962; Kawase A., 1962; Kawase A., 1965]. TAN is also used for the spectrophotometric determination of Cu(II), Cu(I), Rh(III), Os(IV), Complexes having absorbance range from 560 to 620 nm, depending upon the metal ions.

![2-(2-thiazolylazo) naphthol](image)

4-(2′-Thiazolylazo) resorcinol (TAR) was used for the spectrophotometric determination of Cu(II), Bi(III), Pb(II), Zn(II), UO$_2$(II), Tl(III), Co(II), Ga(III), Ni(II). Maximum absorbance was observed from 510 to 570 nm depending upon the metal ions [Havard R. Hovind, 1975].

![4-(2-thiazolylazo) resorcinol](image)

2-(2′-Thiazolylazo)-5-dimethyl aminophenol was used for the spectrophotometric determination of Iron(II) by solvent extraction method with chloroform at pH 8.2 to 10 and the complex was having the absorption maxima at 760 nm [Kazumasa Ueda et al., 1981].

4-(2′-Benzothiazolylazo) resorcinol (BTAR) was used as spectrophotometric reagent for the determination of Co(II) in the pH range of 4 to 6.5 and $\lambda_{\text{max}}$ 510 nm [Ivanov V.M. et al., 1969].
Bromo benzothiazolyl azo naphthol (Br-BTAN), 6-Bromo benzothiazolyl-azo-4-methyl phenol (Br-BTAP) and 2-(2-benzothiazolyl azo)-4,5-dimethyl phenol (BTDMP) were reported as sensitive and highly selective reagent for Cadmium ($\varepsilon = 5.2 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 600 nm in o-Xylene) [Shkrobot E.P. et al., 1966; Gusev S.I. et al., 1971; Dragusin E. et al., 1978].

2-(2'-Thiazolyl azo)-5-dimethyl amino benzoic acid (TAMB) and 2-(2'-benzothiazolyl azo)-5-dimethyl amino benzoic acid (BTAMB) were synthesized. Both the reagents were extensively sensitive chromogenic reagents for the determination of Nickel in the pH range 5 to 9.5. They form 1:1 (ligand: metal) complexes with the maximum absorbance of 610 and 640 nm for TAMB and BTAMB respectively. System confirms Beer's law for 0.05 to 0.5 ppm of Nickel. Interfering metal ions like Copper, Cobalt, Palladium and Iron were masked [Masamichi Furukawa, 1982].

2-(2-benzothiazolyl azo)-5-dimethylamino benzoic acid (BTAMB) was used as a sensitive and selective reagent for the determination of Copper (II) ions in aqueous acetone solution to form a blue complex with a maximum absorption at 650 nm in the pH range 2.5 to 5.0. The complex was stable for
more than 24 hours. Under these conditions, selectivity of the reagent was excellent and most metal ions except Nickel do not interfere when sodium fluoride was added as a masking agent [Takeo Katami et al., 1984]. An extractive spectrophotometric procedure was developed for the determination of Cobalt using 2-(2′-benzothiazolyl azo)-5-dimethyl amino benzoic acid (BTAMB). The absorbance was measured at 705 nm. The method was applied for the determination of Cobalt in paperbush leaves and coal fly ashes, with good precision and accuracy [Take Katami et al., 1985]. 2-[2-(5-Bromo pyridyl) azo]-4,5-dimethyl phenol (Br-PDMP) was synthesized and compared with 2-[2-benzothiazolyl azo]-5-dimethyl amino phenol (BTADAP) for the determination of Cadmium. Br-PDMP and BTADAP dyes were highly coloured 2:1 complexes with maximum absorption at 590 nm and 600 nm. The method was applied for the determination of Cadmium in water samples, plant materials and hair [Kate Grudpan et al., 1989].

Spectrophotometric determination of 2-(2′-benzothiazolyl azo)-4-methoxy phenol (BTAMP) was adopted for the determination of Iron (II). The pH was adjusted from 5.5 to 9.5. Iron complex was extracted in chloroform layer and absorbance was measured at 815 nm. BTAMP Nickel complex shows absorption maxima from 636 nm to 674 nm. Cobalt and Copper complexes shows absorption maxima at 627 nm, 640 nm respectively. Hence the method was applied to the determination of Iron in natural water samples with satisfactory results [Ueda K., 1984].

![2-(2-benzothiazolyl azo)-4-methoxy phenol (BTAMP)](image)

2-(2′-Benzothiazolyl azo) pyrocatechol (BTAPC) was synthesized and used for the spectrophotometric determination of Fe (II) by measuring the
absorbance at 630 nm using borate buffer solution. The method was used in trace Iron detection in Pollens [Feng Jianzhang, 1988].

Mo(IV) with 4-(2-benzothiazolyl azo) pyrogallol was studied spectrophotometrically at pH 4.8 to form 1:2 purple red complex at absorption maxima 572 nm [Zhou Huafang et al., 1997].

\[
\begin{align*}
\text{4-(2-benzothiazolyl azo) pyrogallol}
\end{align*}
\]

Spectrophotometric determination of Cobalt was reported with 2-(2′-benzothiazolyl azo)-5-dimethyl amino phenol (BTAM) [Miao Fengqin et al., 1995]. Determination of Cobalt using the 2-(2′-benzothiazolyl azo)-p-cresol (BTAC) as a spectrophotometric reagent associated with solid extraction was developed. BTAC reacts with Co(II) in the presence of Triton-X100 surfactant forming a green complex with maximum absorption at 615 nm. The reaction was used for the Cobalt determination within a pH range of 6.5 to 7.5, with an apparent molar absorptivity of 1.62 x10⁴ L mol⁻¹ cm⁻¹. A selective procedure was proposed for Cobalt determination in presence of Fe(II), Hg(II), Zn(II) and Cu(II) for milligram levels using masking agents [Marcelo S. Carvalho et al., 1996]. Spectrophotometric determination of Cobalt was done using 2-(6-Methyl-2-benzothiazolyl azo)-5-diethylaminophenol at pH 5 with absorption maxima at 610 nm. By this method, Cobalt in human hair was determined [Tong Yan and Shu Youqin, 2000]. Sensitive chromogenic reagent 5-(2-benzo thiazolylazo)-8-amino quinoline was used for the detection of Cobalt in presence of borax buffer at 650 nm. Method was successfully adopted for the determination of Cobalt in Vitamin B₁₂ and drainage sediment samples [Zhao Shu-Lin, 2002].

Spectrophotometric determination of Zn(II), with 2-(2-Benzothiazolyl azo)-5-sulpho propyl amino phenol with pH 7 at 550 nm was reported and the method was applied successfully for the determination of Zinc in cast aluminium alloy
samples [Ma Weixing et al., 1998] and Zinc determination with 2-(6-methyl-2-benzothiazolyl azo)-5-m-diethyl amino phenol at pH 5, maximum absorption at 602nm. The method was applied to the detection of micro amounts of Zinc in ore with satisfactory results [Tong Yan et al., 2000].

Six new benzoic acid type benzothiazolyl azo reagents were synthesized by introducing some functional groups into the benzene rings, para or meta to carboxyl group and electron donar group-CH$_3$, to 5- or 6-position of benzothiazole ring and were used as spectrophotometric reagents for the determination of Co(II), Fe(III), Ni(II), Cu(II) and Pd(II) and the results showed that different substituted groups affect the sensitivity and selectivity of these reagents. Among the reagents 5-Me-BTAEB was most sensitive [Zhang Jiaoqiang et al., 1999].

1-(6-bromo-2-benzothiazolyl azo)-2-naphthol was used as a spectrophotometric reagent for the determination of Cadmium, Cobalt, Manganese and Copper at pH 11 [Gao Hong-Wen et al., 2001].

![1-(6-bromo-2-benzothiazolyl azo)-2-naphthol]

4-(2’-Benzothiazolyl azo) salicylic acid (BTAS) was used as a reagent for the simultaneous first derivative spectrophotometric determination of Ni(II) and Fe(III). Reaction of BTAS with Nickel provides the formation of a complex with an absorption peak at 525 nm, and with Fe(III) absorption peak at 511 nm. Their proposed method was successfully applied for the determination of Nickel, Iron, Steel and Aluminum alloys [Hashem E.Y. et al., 2003].
4-(2'-Benzothiazolyl azo) salicylic acid (BTAS)

7-[6-Methoxy (2-benzothiazolyl azo)]-8-hydroxy quinoline-5-sulphonic acid (6-Meo-BTAQS) and 2-(6-methyl-2-benzothiazolyl azo)-5-diethyl aminophenol were reported for the spectrophotometric determination of micro amounts of Nickel [Xia Xinquan et al., 1998; Tong Yan et al., 1998]. Spectrophotometric determination of the Nickel with 5-(6-nitro-2-benzothiazolyl azo)-8-aminoquinoline showed the maximum absorption at 615 nm. 5-(2-Benzothiazolyl azo)-8-amino quinoline was also used as reagent for the determination of Nickel with the maximum absorption at 610 nm. Both these reagents were used for the determination of trace amount of Nickel in aluminium alloys [Chen Ju et al., 1999; Zhao Shulin et al., 2001].

New thiazolyl azo chromogenic reagents 8-[2-benzothiazolyl azo]-1-naphthalene diol and 1-[2-benzothiazolyl azo]-2,3-naphthalene diols were synthesized and were used as chromogenic reagents for the determination of Nickel. This method was adopted for the spectrophotometric determination of Nickel in tea leaves, papper bush and natural water samples [Amin A.S., 2001].

Spectrophotometric determination of Copper was carried out using 2-(2'-benzothiazolyl azo)-5-sulphoproyl amino phenol at pH 7. Complex showed maximum absorption at 562 nm and Copper detection was done in Aluminium alloys [Ma Weixing et al., 1999]. 2-[2-(6-methyl benzo thiazolyl) azo]-5-diethyl amino benzoic acid was used for the detection of Copper with pH 3 to 6.5 at 655 nm. This method was used for the detection of Copper in aluminum alloy and industrial wastewater [Qui Fengxian et al., 1999]. 3-(Benzothiazolyl azo)-2,6-dihydroxybenzoic acid used for the spectrophotometric determination of Copper at pH 6.98 with maximum absorption at 547 nm [Guo Ancheng et al., 2000]. 2-[2'-(6-methyl-benzothiazolyl azo)]-4-bromo phenol was used as a sensitive
reagent for the determination of Copper. Using this technique river and well water samples are analysed for Copper [Lemos Valfredo A. et al., 2006].

4-(2-Benzothiazolyl azo)-2,2′-biphenol, 5-(2-benzothiazolyl azo)-8-hydroxy quinoline, 1-(2-benzothiazolyl azo) 2,3-naphthalene diol and 8-(2-benzothiazolyl azo) 1,4-naphthalene diols were used for the spectrophotometric determination of Mercury at wave lengths 590, 629, 606 and 591 nm respectively. The method was used for the detection of Mercury in environmental, human Urine and blood samples [Amin Alaa et al., 2000].

2-(Benzothiazolyl azo)-7-(4-carboxy phenyl azo) chromotropic acid was synthesized and used for the spectrophotometric determination of Iron(III) at maximum absorption at 685 nm [Zhang Xuejun et al., 2003].

Table 4.2 shows analytical characteristics and spectrophotometric procedures for the metal determination using benzothiazolyl azo reagents.

![2-(benzothiazolyl azo)-7-(4-carboxy phenyl azo) chromotropic acid](image-url)
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Analyte</th>
<th>pH</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Linear range $\mu$g mL$^{-1}$</th>
<th>Detection limit $\mu$g mL$^{-1}$</th>
<th>$\varepsilon \times 10^5$</th>
<th>sample</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-(2-Benzothiazolyl azo)-2-hydroxy-3-naphthoic acid</td>
<td>Pd (II), Nb (V)</td>
<td>4.5</td>
<td>677 649</td>
<td>20-1200 50-2200</td>
<td>-</td>
<td>0.73 0.34</td>
<td>-</td>
<td>Ores</td>
</tr>
<tr>
<td>2-(2-Benzothiazolyl azo)-3-hydroxy phenol</td>
<td>U (VI)</td>
<td>5.99</td>
<td>599 50-1700</td>
<td></td>
<td></td>
<td></td>
<td>Standard ore and geological samples</td>
<td>Amin A.S and Mossalamy E.I-E.H 2003</td>
</tr>
<tr>
<td>2-(2-benzothiazolyl azo)-4-methoxy phenol</td>
<td>U (VI)</td>
<td>608</td>
<td>50-1400</td>
<td></td>
<td></td>
<td></td>
<td>Standard ore and geological samples</td>
<td>Amin A.S and Mossalamy E.I-E.H 2003</td>
</tr>
<tr>
<td>2-(2-benzothiazolyl azo) phenol</td>
<td>U (VI)</td>
<td>585</td>
<td>50-1600</td>
<td></td>
<td></td>
<td></td>
<td>Geological samples</td>
<td>Amin A.S and Mossalamy E.I-E.H 2003</td>
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<td>2-[2-(6-methyl benzo thiiazolyl) azo]-5-(N-methyl-N-sulpho methyl)</td>
<td>Ni (II)</td>
<td>4.5-6.0</td>
<td>620 0-240</td>
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<td>0.82</td>
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<td>0.88</td>
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<td>2-[2-(6-methyl benzo thiiazolyl) azo]-5-(N-ethyl-N-carboxy methyl)</td>
<td>Ni (II)</td>
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<td>4-(2-Benzothiazolyl azo)-2,2-biphenyldiol</td>
<td>Nb (V)</td>
<td>5.0</td>
<td>612 50-2500</td>
<td>15.0</td>
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<td>0.28</td>
<td>Ores</td>
<td>Amin A.S, 2000</td>
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<td>4-(2-Benzothiazolyl azo)-3-hydroxy-2-naphthoic acid</td>
<td>Ni (II)</td>
<td></td>
<td>643 50-3100</td>
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<td>Biological samples</td>
<td>Amin A.S, 2001</td>
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<tr>
<td>5-(Benzothiazolyl azo)-2,5-naphthalenedioli</td>
<td>Pd (II), Nb (V)</td>
<td>4.0</td>
<td>638 661</td>
<td>20-1350 50-1700</td>
<td>15</td>
<td>0.62 0.45</td>
<td>Ores</td>
<td>Ahmed I.S, 2005</td>
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<td>2-[2-[5-Methyl benzothiazolyl azo]-5-dimethyl amino benzoic acid</td>
<td>Ni (II)</td>
<td>5.6</td>
<td>640 0-400</td>
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<td>1.32</td>
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<td>Benzothiazolyl diazo amino azo benzene</td>
<td>Ni (II)</td>
<td>9.4</td>
<td>550 0-280</td>
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<td>1.96</td>
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<td>Shulin Z et al., 1997.</td>
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4.5 CONCLUSIONS

Pyridylazo, Thiazolylazo, benzothiazolyl azo dyes were widely used for many analytical procedures like solid phase extraction, Liquid chromatography, liquid-liquid extraction, cloud point extraction, electrochemical methods etc. However, these reagents appear yet to have great potential for application in preconcentration systems. The wide spectrum of potential methods for inorganic and organic substances makes Pyridylazo, Thiazolylazo, Benzothiazolyl azo dyes as powerful reagents for analytical measurements. Due to the good analytical characteristics obtained, such as detection limit, enrichment factor and precision of the proposed procedures involving Pyridylazo, Thiazolylazo and Benzothiazolyl azo compounds have been demonstrated to be promising for trace element analysis [Valfredo Azevedo Lemos et al., 2007].

Benzothiazolyl azo dyes are selective and sensitive reagents for spectrophotometric analysis. Therefore we planned to synthesize some new reagents by diazotizing 2-amino benzothiazole and its 4-methyl, 6-chloro, 6-bromo derivatives and couple the respective diazo salts with 2-amino-3-hydroxy pyridine. These new benzothiazolyl azo dyes were used as reagents for the spectrophotometric determination of Copper, Cobalt, Cadmium, Nickel and Zinc. The proposed method was successfully applied for the determination of metal ions from the industrial effluents.