Ministry of Higher Education and Scientific Research
University of Babylon

SYNTHESIS, STUDYING AND ANTIMICROBIAL ACTIVITY OF SOME NEW AZO DYES AND SOME OF THEIR METAL IONS COMPEXES

A Thesis
Submitted to the Council of the College of Science, The University of Babylon,
As a Partial fulfillment of the Requirements for the Degree of Ph.D. of Science in Chemistry.

By

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Ph.D.

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H. A. Salih

Dedication

To
The memory of my father,
My mother and brothers,
My patient wife, Bushra,and
My dearest and most beloved
Sahar, Taha, Mais and Aows.

Certificate

We certify that this thesis was prepared under our supervision at the Department of Chemistry, College of Science, University of Babylon, as a partial requirement for the Ph.D. degree in Chemistry and that this work has never been published anywhere.

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Abstract

The work described in this thesis was undertaken at the University of Babylon between October 1999 and May 2002 under the supervision of Dr. Ali Muhammad Al-Rikabi and Dr. Jalil Raheef Ugal. Except where indicated by references, it is the original work of the author and has not been submitted for any other degree.

This work deals with the preparation and identification of three series of azo dyes, namely, benzothiozol-2–ylazo(BTZ),benzimidazol-2-ylazo(BIZ)and2H-(1,2,4triazol-3-ylazo)(TZ)derivatives.

The characterization of these reagents was performed by elemental analysis (CHN) mass spectra, Uv – vis. and IR spectral data. The investigation was performed to determine the ionization constants of the ligands under consideration potentiometrically in 30% v/v dioxane-water, the substituent effect on pK_a also examined, and it was shown there were influenced by coordination of the ligand to the metal. The decrease in pK_a of these ligands was an indicative of the extent to which coordination could increase the acid strength of such ionizable atoms or groups.

The stability constants of the complexes Produced from the reaction of the above ligands with (UO_2(II), Pb (II), Ce(III), La (III), In (III), Zn (II), Cu(II) and Ni(II)) were determined spectrophotometrically in 30% v/v dioxane-water to show the long range of sensitive of the ligands above , The temperature effects on the stability constants of these complexes were also studied, and they were found to decrease with increasing the

temperature. The thermodynamic functions ($\Delta G^{o}, \Delta H^{o}$ and ΔS^{o}) were calculated experimentally. However, the electron donating substituent increased the electron density around chelating groups and consequently increased the stability of the complexes. Many factors effecting the resulting parameters were also explained.

The present work indicates that it is possible to make a more detailed discussion of the nature of the bonds formed between the ligand and the metal .The ΔH^{o} value reflects the changes in the numbers and strengths of bonds made and broken during the reactions, It is interesting to relate the values of ΔH^{o} to the type of bonding between the metal ions and the ligands molecules and to the structural features of the complexes. The separation of ΔH^{o} into electrostatic and covalent components was therefore, done.

It has been observed that some of these azo dyes have invitro anti-bacterial activities against three types of bacteria (*E.coil, Stap. aureus and Ps. aerogenosa*) .

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Nomenclatures

Physical Notation		***Abbreviations***	
k_a	Ionization constant	PAN	4(2-pyridylazo)-1-naphthol
K_{ML}	Stability constant	IR	Infra – red
T	Absolute temperature	PAR	4(2-pyridylazo)- resorcinol
G	Gibbs ' free energy	TAR	4(2-Thiazolylazo)-2-resorcinol
μg	Micro gram	TAN	1(2-Thiazolylazo)-2-naphthol
R	Gas constant	TLC	Thin Layer Chromatography
ε	Molar extinction coefficient	Uv.-Vis.	Ultraviolet – visible spectroscopy
v	Molar volume	NMR	Nuclear Magnetic Resonance
f_x	Activity coefficient of component (x)	BTZ	Benzothiazolyl -
K_{OH}	Hydrolysis constant of metal ion	BIZ	Benzimidazolyl-
r	Radius of atom	TZ	Triazolyl-
Z	Charge of electrons	VBT	Valance Bond Theory
H	Enthalpy	CFT	Crystal Field Theory
S	Entropy	MOT	Molecular orbital theory
ΔH_e	Electrostatic enthalpy change	LFT	Ligand Field Theory
ΔH_c	covalent enthalpy change	MIC	Minimum inhibitor concentration
I	Ionic strength	NCCLS	National Committee for Clinical Laboratory Standard (U.S)
kJ	Kilo Joul	R_f	Rate of flow
		8.HQ	*8.hydroxy quinoline*
		E.coli,	*Escherechia coli,*
s	Sharp Pick	*staph. aureus*	*Staphylococcus aureus*
b	Broaden Pick	*Ps. aergenosa*	*Pseudomonas aergenosa*

Chapter One

Introduction

1.1 General Introduction :

The first systematic study of complex compound was carried out by Werner [1]. He published a purely empirical theory, and developed idea that most metals possessed two types of valences: (a) primary valence, which is called oxidation state, that satisfied the chemical equivalence of the atom, (b) secondary valence, satisfied by the coordinated atoms. Metal ions tend to satisfy both valences.

In a complex, there are a number of groups bond directly to the metal ion (this number is now referred to as the coordination number), and also there are ions present of opposite charge to that of the complexes metal ion, the number of ions being sufficient to neutralize the charge of the complex ion. Werners` theory explained the stereo-chemistry and isomerism of complexes, but it failed to explain why certain elements had a greater tendency to form such compounds than others.

Lewis [2] explained the formation of a coordinate bond according to the interaction between the metal ion and the lone pair of electrons on the ligand.

Using valance bond theory (VBT) and the concept of orbital hybridization, Pauling[3] suggested that in order that the ligand should donate the electron pairs , the atomic orbitals on the metal ion should be hybridized in such as to supply equivalent orbitals with the required symmetry properties which explain the nature of the bonding that occurs in

these complexes. He used the magnetic moments of these compounds as a criterion of the geometry.

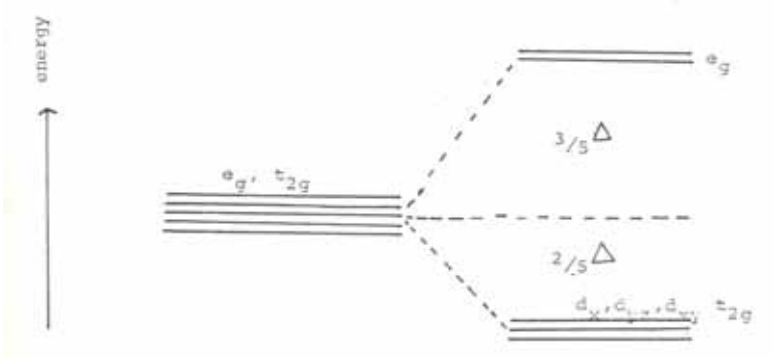
For example, $[FeF_6]^{3-}$ has a magnetic moment, which corresponds to five unpaired electrons whereas $[Fe(CN)_6]^{3-}$ has a moment which corresponds to one unpaired electron , Pauling suggested that $[FeF_6]^{3-}$ was an ionic complex whereas $[Fe(CN)_6]^{3-}$ was a covalent one . A coordinated bond can be formed between any atom or ion which can share in a pair of electrons (acceptor), and another atom or ion can furnish a pair of electrons (donor)[2].

VBT, however, failed to give full or adequate explanation of certain aspects of the electronic spectra and magnetic properties of complexes, which assumed that all **3d** orbitals had the same energy.

Recently, the ligand field theory (LFT) has been widely used for the interpretation of the structures and properties of the complexes. This incorporates the crystal field theory (CFT) of Bathe and Van Vleck [4]; which assumes that the bonding in the complex is due to the electrostatic (ionic) interaction between the positive nucleus of the metal cation and the negatively charged electron of the ligand.

CFT considers the effect of an electrostatic field on the degenerate **d** orbitals of the cation. With the aid of this theory, the separation of energies of many groups was brought about in a way depending on the magnitude, symmetry, and the oriented dipoles applied on the perturbing field forces.

Scheme (1-1)



LFT also incorporates molecular orbital theory (MOT) in its conclusion which follows same traces of the bonding occurring, which the molecular orbitals are formed from the **d** , **s**, and **p** orbitals of the central ion and the ligands. Atomic orbitals which belong to the same symmetry class can combine . (LFT) assumes that the ligands electrons not only impose electrostatic fields on the metal ion, but also allow the possibility of covalent character, the ligand field effects the energies of the metal orbitals, **s** orbitals being effected equally in all directions. The effect of the ligand field on d orbitals is more complicated. In octahedral configuration, the $\mathbf{e_g}$ set of orbitals, that is $\mathbf{dz^2}$, $\mathbf{dx^2\text{-}y^2}$ orbitals, are directed along the cartesian coordinates, whereas the $\mathbf{t_2g}$ set, that is the **dxy,dxz** and **dyz** orbitals, are directed between the axis . Ligands are assumed to approach the metal ion along the Cartesian axis, In this case, the d orbitals split in two groups of orbitals separated in energy by (Δ) the value of Δ depends on the field strength of the ligand; for example, for a given metal ion the ligands may be arranged in order of increasing Δ or field strength.

Molecular orbital theory (M.O.T) Suggests that the metal-ligand bonding occurs by overlap of atomic orbitals of the metal ion and the molecular orbitals of the ligand, providing that they have the same symmetry and similar energies. So that for transition metal ions have only five valance orbitals their lobes pointing towards the corners of an octahedron ($dx^2\text{-}y^2$,dz^2, p_x , p_y and p_z) in addition to (s) valance orbital. The valance orbitals of the ligand are (s) and (p) atomic orbitals or hybrids of these orbitals.

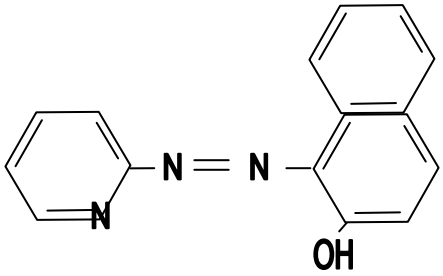
For an electrostatic field of cubic symmetry by six negatively charged ions, the combination of atomic orbitals leads to six bonding and

six anti bonding molecular orbitals, There are no ligand orbitals available belonging to the same class as the **t_2g** orbitals of the central cation and so remain as non-bonding orbitals; whereas the **eg** orbitals combine within to give a doubly degenerate bonding and a double degenerate anti-bonding orbitals. Further, it has been shown that the **t_2g** orbitals have π – bonding the correct symmetry for the formation of π- bonds. i.e π- bonding may occur when orbitals of suitable symmetry are available on the ligand molecule .

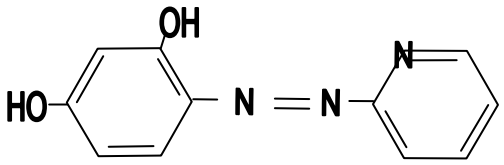
1.2 Azo Dyes:

The earliest works on the heterocyclic dyes were concerned with 8-hydroxy quinoline (8-HQ) as a ligand, This compound was used to detect many cations[5], whereas Cheng and Bray[6] investigated the reactions of 1-(2-pyridylazo)2-naphthol (PAN) with certain metal ions . They recommended this reagent may used as an indicator and the observing that the chelate complex of PAN might be extracted by organic solvents.

Wehber [7] used 4-(2-pyridyl) resorcinol (PAR) as a reagent, claiming it to be superior to PAN, because of the good solubility of PAR and its chelate effect in water compared with PAN.



PAN



PAR

Scheme (1-2)

Pollard *et al*. [8] used the azo dye PAR as a spectrophotometric reagent for measuring Co (Π), U(VI) and Pb(Π).also PAR has been used in the analysis for about fourty different metal ions.

Two reviews included all dyes containing a heterocyclic group ortho to the azo group, synthesized from (8-HQ) derivatives, concerning analytical applications of these heterocyclic azo dyestuffs and closely related compounds, was were published by Navratil [9] and by Anderson and Nickless[10]. A survey of the azo dyes prepared from 2-amino thiazole and 3-amino triazole derivatives was done by Hovind [11] . A large number of the derivatives of these two compounds have been investigated for analytical purposes , and have been used as reagents in addition to being interesting complexing agents [12-14]. Recent papers concerned with a novel benzothiazole and triazole derivatives reported their ability to form a chelate with many metal ions [15-18] .

The synthetic methods of benzimidazole and closely related heterocyclic compounds have been reviewed in details in Grimmett volumes A, and B [19] which contain a wealth of tabular material, including instruments of modern safety considerations.

1.3 Metal *I*ons in Solution:

Ionic salts have a large lattice energies, therefore, a considerable energy is required in separating their constituent ions when they dissolved so to give solution containing discrete cations and anions dispersed throughout the solvent, the solvation of ions into a polar solvating, solvent

is advantageous from the point of view of the ions with pronounced inter molecular forces, such as dipolar interaction and hydrogen bonding [20].

Aquo-cations, especially those of 3+ and small 2+ ions tend to act as acids in solution

$$M(H_2O)_X^{+n} \text{---} M(H_2O)_{X-1}OH^{(n-1)} + H_{eq}^{+}$$

This occurs in mixed and non-aqueous solvents. Such phenomena are observed mostly of protic ligands. To determine the stability constants of metal ligand complexes, the hydrolysis constant pK_{OH} of aquo cation introduced, must be known, owing to avoid the hydrolysis and polymerization during the complex formation process [21].

Values of $pk_{(OH)2}$ for the loss of a second proton from an aquo-cation are 10 to 100 times smaller than that for the first proton

1.4 Metal Ion Complex Formation in Solution:

When a metal ion combines with an electron donor ligand, the resulting substance is a complex or a coordinating compound.

When a ligand contains two or more donor atoms and were able to bind to a metal to form heterocyclic ring with the metal, the resulting structure is a “chelate compound”, while if two metal ions more bonding to gather with ligand the compound is a ”bridge complex”.

Bjerrum [22] postulate that complex formation in solution occurs in a stepwise manner, each step was described by a separate stepwise formation constant. When a metal (M) reacts with a ligand (L) in aqueous solution, the reaction involves the replacement of water molecules by ligand

molecules in the coordination sphere of the metal ion, which is represented by:

$$\left[M(H_2O)_n\right] + L \longrightarrow \left[M(H_2O)_{n-1}L\right] + H_2O$$

The equation of the equilibrium between the metal and ligand is written as :

$$M + L \longrightarrow ML$$

$$K_{ML} = \frac{\left[ML_1\right]}{\left[M\right]\left[L\right]} \qquad \text{.....(1.1)}$$

For the stepwise replacements, the stepwise equilibrium constants can be written:

$$K_{MLn} = \frac{\left[ML_n\right]}{\left[ML_{n-1}\right]\left[L\right]} \qquad \text{.....(1.2)}$$

where K_{ML} , K_{ML2} ……K_{MLn} are the stepwise formation constants, The overall formation constant of ML_n may be written:

$$\text{ß}_n = \frac{\left[ML_n\right]}{\left[M\right]\left[L\right]^n} \qquad \text{……..(1.3)}$$

Determination of stability constant is of primary importance in the study of complex compounds, since it is the most promising approach to the accumulation of data which should be approached to understand the relationship which govern their formation and structure [(23)], It is one of the most important evidence for the existence of complexes in solution.

There are many factors affecting the magnitude of stability constant, which can be summarized as follows:

1.Chelate Effect:

A multidentate ligand generally formed a more stable complex than that of monodentate ligand, which is can be related to entropy effects [24], The chelate effect (γ)expressed in terms of:

$$\gamma = \log K_{ML} - \log K_{MA} \qquad \text{.....(1.4)}$$

where K_{ML} is the stability constant of the chelate complex, and K_{MA} is the stability constant of non the chelate complex.

For chelation to occur, the ligand must contain at least two donor atoms, which are able to bind to the same metal in a way that the chelate ring is sterically possible; the stability of the chelate complexes tends to be high. [23]

2. The Nature of Metal Ion :

Metal ions behave as Lewis acids and ligands as Lewis bases. The terms "hardness" and "softness" have been used to describe the interaction between the metal ion and the ligand .A "hard" metal ion is one which can retain its valence electrons very strongly, a "soft" metal ion is relatively large and does not retain its valence electrons strongly.[25]

The Z/r of the metal ion could effect the stability and geometry of the complex [26]. The most stable complexes are those which have their cations derived from the transition metal and metals that behave like transition metals. The stability order was established by Irving and Williams, which is valid for most nitrogen or oxygen donor ligands with a coordination number of four irrespective of the nature of ligands. The order $Mn^{2+} < Fe^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$ was justified by Irving and Williams[27]

by comparing the ionic radii and the second ionization energy of the metal ions.

3.The Nature of the Ligand:

The radius and charge of the donor atom are important factors affecting the electrostatic interaction. With conjugated ligands possessing low lying unoccupied molecular orbitals ; using by anti bonding molecular orbitals, a back-donor of electrons from filled metal **d** orbitals to these empty ligand molecular orbitals may occur. The resulting double bond character leads to an increase in the bond strength and complex stability[28].

4. The Basicity of the Ligand:

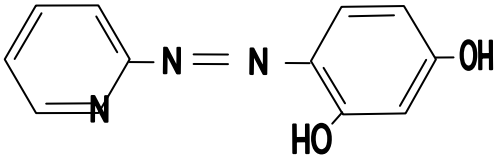
In the majority of cases, a complex formation means competition between the metal ions and protons, a correlation between $\log K_{ML}$ and pK_a of the protonated ligand might be expected. Many workers [19-31] have confirmed that the more basic the ligand tends to be, the higher metal ligand bond, whereas the presence of conjugation or steric hindrance or π-bonding alters this factor. Other factors affecting stability constants are, steric effects and statistical considerations Nakamoto volume A [32].

1.5 Stability Constants, Thermodynamic and Structural Studies of Azo Dyes Complexes with Metal Ions:

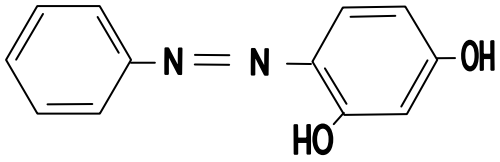
Complexes of various metal ions have been studied in solution as well as in the solid states by many workers[32](volume B).

Geary *et al*. [33]. measured the stability constants of metal chelates complex for the analogous ligands, PAR, benzene azo resorcinol,

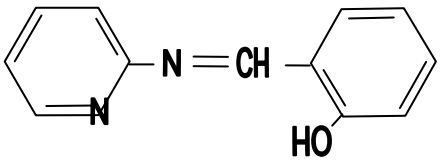
salicylidene 2- amino pyridine and 2-pyridene-o-aminophenol as shown in scheme (1- 4) . They concluded that 2- pyridylin-o-phenol chelates were in the same order as those of PAR; The chelation with metals occurred through the pyridine nitrogen atom , the azo nitrogen atom nearest to the phenolic part of the molecule and the o- hydroxyl group which gave rise to two stable five membered chelate rings .



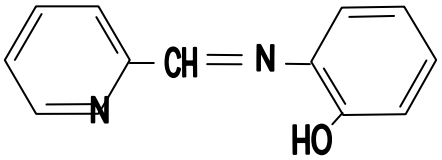
PAR



benzene azo resorcinol



Salicylidene 2-amino pyridine



2-pyridyline – o -amino phenol

Scheme (1-4)

Wada *et al.*[34] made a complete investigation of the stubility constants of 1-(2-thiazolylazo) –2- hydroxy-3-naphthoic acid (THNA). As a metallochromic indicator, the dissociation constants of (THNA) were determined as a function of dioxane percent of aqueous solution which shows that the pK_a of both (COOH) and (.OH) groups increase with increasing the percent of dioxane. The formation constants of this ligand chelates of copper, nickel and zinc were also obtained in (μ= 0.1, 25C^o, in 5% v/v dioxane water),and followed Irving William series .

Table (1-1) Acid ionization constants of THAN

pK_{COOH}	pK_{OH}	%(v/v)dioxane - water
3.4	9.83	5
3.6	10.4	20
3.9	11.5	50

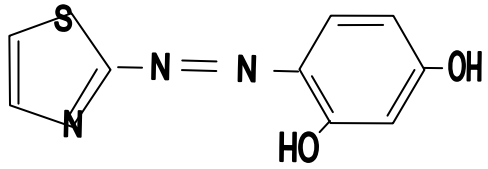
The interaction of benzoxazole –2-thione with Cr(III), Cu(II), Cu(I), Zn(II), Cd(II), and Hg(II) have been studied by Carlo and Tosi [35]. The complex formed were characterized on the basis of analytical conductivity, U.v-Vis, spectral IR and magnetic data. A detailed discussion on the analysis of the position and intensities of the thioamide bonds have been carried out in order to distinguish the coordination center of the ligand. A normal 1:1 complex with that metal ion has been obtained.

The reaction of 4-amino –3-5- dimercapto 1-2-4 traizole with some divalent metal ions (Ni(II),Co(II),Cu(II),Pd(II),Zn(II),Cd(II) and Pb(II)) have been studied by Mishear[36]. The characterization of complexes by different physicochemical methods were approved. Intermolecular hydrogen bonding was also invisigated.

4-(2-pyridylazo) resorcinol (PAR) constitutes a selective reagents for the spectrophotometric determination of Zn (II), the formation constant of the (1:1) PAR metal complex was determined, by using nitrilotriacetic acid as a competition ligand [37] .

Ohyoshi[38] has shown that by using reverse order method, the values of stability constants of (PAR) with Mn (II), Cu (II) and Zn(II) complexes are higher than that of 4-(2-thiozolyazo) resorcinol (TAR), Scheme(1-5). With some metal ions, (TAR) forms less stable complexes with metal ions

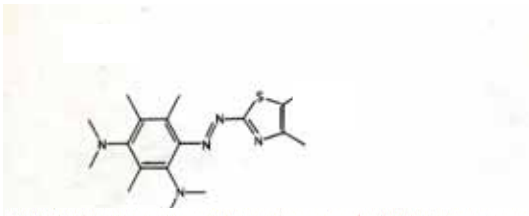
than (PAR) because of the low basicity of thiazole rings. The difference in behavior of **3d** transition metals (Π) compared to the lanthanides (Ш) was also discussed.



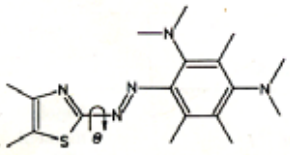
Scheme (1-5)

Truj *et al.*[39] studied the complex equilibrium in the system Pd(Π) – 4(5- - methyl – 3- isoxazolylazo)- resorcinol by spectrophotometric method Many interesting correlation regarding the stabilization of these complexes were discussed.

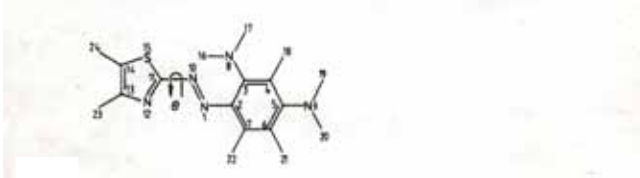
In order to evaluate the preferred molecular configuration of these azo dyes, a theoretical study [12] on the molecular structure of 4-(thiozolyl azo) benzen diamine shows that there are three possible structures A, B, and C are employed, as shown in Scheme(1-6) The B structure is supported as the most probable form according to the energy calculation, dipole moments, and the enthalpy of formation [142]



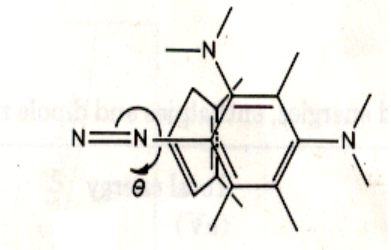
1. The proposed structure of 1-3 benzenediamine,4-(2-thiazolylazo).



. *Trans* 1-3 TADAB: structure **B** (θ=0°).



Trans 1-3 TADAB: structure **A** (θ=0°).

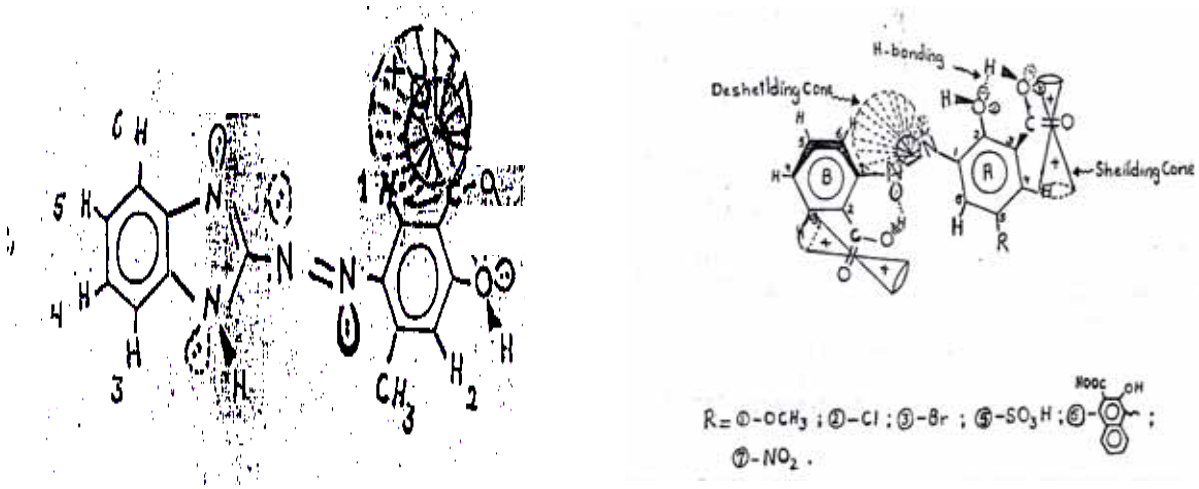


Cis 1-3 TADAB: structure **C** (θ=90°C).

Scheme (1-6)
[From ref.(12)]

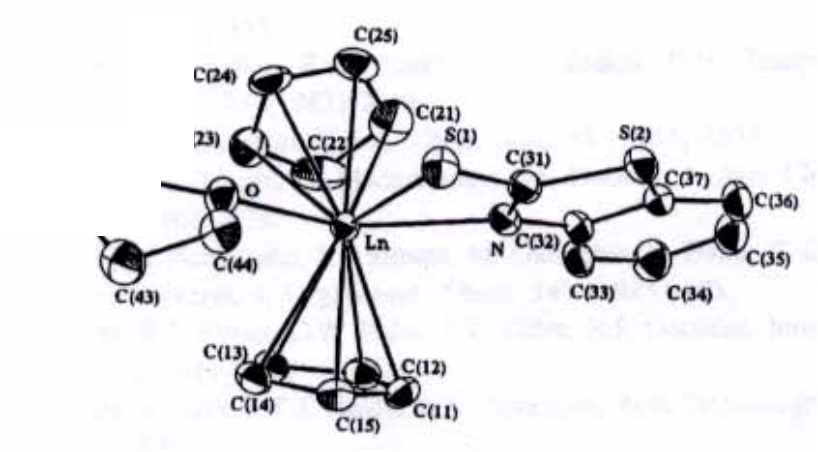
A series of studies regarding many azo compounds were reported by many workers [32], using IR, and Raman spectroscopy, CHN analysis and H-NMR. The conformations were proved as preferred flattened chair – chair structures which give good results toward the complexometric abilities of these ligands toward the metal.

Oda *et al.*[42] studied the molecular structures of many azo dyes using IR and H.NMR spectroscopy as seen in scheme (1-7) . A remarkable difference between the chemical shift and coupling constants values was shown, which were attributed to two main factors. The first one is the possible intermolecular hydrogen bonding interaction between the lone pairs of electrons on the hydroxyl, acetyl groups, and the repulsion of the lone pairs of electrons on the azo group with those on the hydroxyl, acetyl groups and with the unpaired electrons on the heterocyclic group, which cause a certain rotation of the two rings leading to a zig – zag configuration. The second factor is the variation in the attachment position of linking of the azo group to the benzene ring.



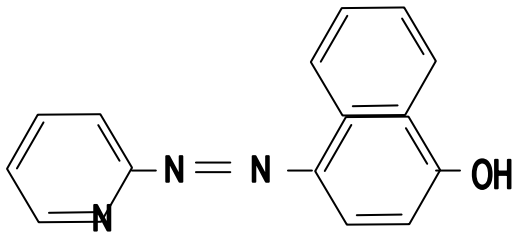
Scheme (1-7) [From ref.(42)]

Zhang *et al.*[43] made a reaction between equimolar amounts of 2 – mercapto benzothiozole and some lanthanide ions at room temperature to form a new trigonalbipyramidal geometry, scheme (1-8) with a coordination number of five. Analogous complexes were studied by other workers [44 -54].



Scheme (1-8) [From ref.(43)]

An investigation of metal complexing properties of 4-(2-pyridylazo)-1-naphthol)p-PAN scheme(1-9)was reported by Better *et al.* [55]



Scheme (1-9) [From ref.(55)]

shows that it is a strong complexing agent when loses a proton from the hydroxyl group. A comparison was made with another ligand 1-(2-pyridylazo)-2 naphthol, the latter was shown to be a stronger one due to the presence of hydroxy group in the ortho position assuming that it might be interacted with as a tridentate complex.

A Calorimetric studies on 4(5)(2- aminoethyl imidazole and 2-(2-aminoethyl imidazole) were reported by Eilbeck *et al* [56]. Enthalpy and entropy data were obtained for the formation of their copper complexes, and the values for the formation constants were obtained under conditions of high ligand /metal ratio, where hydrolysis may be neglected and corrected, while another work [57] indicating the greater stability of 4- (2-pyridylazo) imidazole metal complex comparing with those of 2-(2-pyridylazo) 2-(2-pyridylazo) imidazole, which might be explained on the basis of to the steric hindrance of 2-(2-pridylazo) imidazole which was destroyed on complexation.

Thermodynamic functions for the formation of various of metal complexes with nitrogen and carboxylate containing ligands in aqueous solution were analyzed in terms of temperature – dependent and temperature independents parts [58]. A method for the calculation of the temperature – independent component of the enthalpy change (ΔH_c) was described in terms of M- N and M – O covalent interactions. Specific steric requirements of thermodynamic data for a series of aminocarboxylic ligands indicating an appreciably more exothermic, (ΔH_c) as compared with pure nitrogen ligands. It is clear that the contributions made by carboxylic oxygen coordination are not negligible; in addition, the structural requirements for stable five – members chelate rings are more readily met when both nitrogen and oxygen atoms are contributing in a complex formation [59].

A chelation of a series of 1 – pyrazolyl borates with some divalent transition metal ions in water and a cetonitrile solvent has been studied by Jezorek and Mc.Gurdy [60]. The thermodynamics of chelation of Co(II),

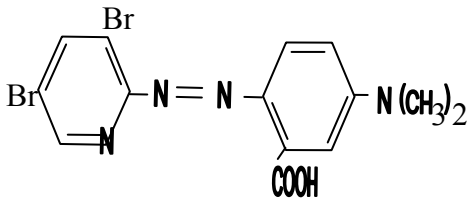
Ni(II) and Zn(II) with four poly (1- pyrazolyl) borate ligands was reported in water and acetonitrile media, whereas the precipitation of the chelates occurs in all cases in water. All product species were soluble in acetonitrile. Enthalpy results suggested that six – coordinate Co (II) complexes may be tetragonal rather than octahedral. Evidence is presented indicate the formation of six – coordinate, anionic chelates of Co (II) and Ni (II) with the bidentate, monoanionic dihydro bis (1-pyrazolyl) borate ligand in acetonitrile solution. The substitution of a methyl group in the pyrazole rings of this ligand prevents chelate anion formation as a result of steric hindrance. Enthalpy values for the substituted ligand were lower than for the unsubstituted one in those cases where a steric effect is expected. Replacement of the remaining boron hydrogen of hydrotris (1-pyrazolyl) borate by a fourth pyrazole group results in substantially decreased enthalpies for reaction in both water and acetonitrite media, most likely, because of coordination compilation with the uncomplexed pyrazole ring. The larger enthalpy values obtained in acetonitrite in all cases were attributed to the markedly weaker salvation of the metal ions and ligand by this solvent than by water.

Siefker and Aroc [61] used the spectrophotometric data to determine log K_{ML} and ε for complexes of Cu (II) with N – methyl diethanol amine, 1,4-bis(2–hydroxypropayl)–2-methyl piperazine and 2–amine methyl-1-propanol. Values of log K_{ML} of these complexes were reported to be log K_{ML} = 5.22 , 5.41 and 5.38 respectively. Whereas Douheret [62] used potentiometric and polarographic methods to determine equilibrium constants (log K) for complexes of Cu(II) with some N – substituted ethanol amines.

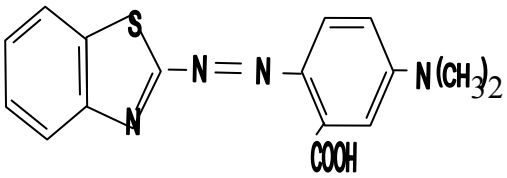
A spectrophotometric method was applied by Ohyosh [63] , to determine the formation constants of various types of complexes using another competating ligand. This method has been developed for the determination log K_{ML} of various colored complexes ML and ML_2, and measuring the absorbance of all forms of colored complexes in the absence and presence of another ligand Y. A method was employed on the competition between two ligands, L = PAR and Y = nitrilo triacetic acid so the formation constants of various types of colored complexes were obtained using the linear – squares method. The formation constant of Zn – PAR was reported as follows: -

$$\log K_{M(HL)_2} = 9.66 \quad \log K_{M(HL)L} = 15.89 \quad and \log K_{ML_2} = 21.52$$

Kamati *et al.*[64] synthesized2-(3,5-Dibromo pyridylazo)-5- dimethy laminobenzoicacid(PAB)Scheme(1-10)and2(2-benzothiazolylazo)-5-di methylamino benzoic acid (BTB)Schem(1-10), Sensitive organic reagents with various transition metals. Spectrophotometric method has been used for the determination of trace amounts of cobalt, copper, iron, nickel, palladium and vanadium in fuel oil and pond sediment using these reagents. Acid ionization constants and the stability constants of metal complexes of the these in reagents 40% (v/v) dioxane solution were determined [65-66].



PAB (pK_a = 5.61)



BTB (pK_a =4.91)

Scheme (1-10)

The probable stability constants of PAB and BTB with Cu (II) V (IV), Pd (II) and Zn(II) were reported in Table (1-2) :-

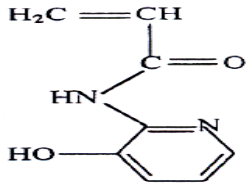
Table (1-2) : Stability constants of PAB and (BTB)

Metal	Log K_{ML} (PAB)	Log K_{ML} (PAB)
Cu(II)	10.73	7.83
V(IV)	8.97	6.62
Pd(II)	10.36	9.37
Zn(II)	6.27	----------

The complexation equilibria between Cd (II) and 4(4\ - methyl - 2\ . thiazolylazo)–2 methyl–resorcinol were studied by Sanchez *et al.* [67] , using spectrophotometer technique in 50:50 ethanol – water solvent . A graphical and numerical calculation methods were used to obtain acid equilibria of the reagent and its complexation equilibria with Cd(II) were also reported, thus at a given pH range, the ionic strength and the presence species in a solution, pK_{a1}= 6.49, pK_{a2} = 11.07 and log K_{ML_2} = 11.83.

Proton ligand ionization and metal stability constants of 2-acryl amido –2-amino-3- hydroxy pyridine (AAHP) scheme (1-11) with Mn (II), Co (II), Ni(II), Cu(II), La(III) and Th(IV) metal ions in 0.1M KCl and 50% (v/v) ethanol – water mixture were calculated potentiometrically by

Ashraf *et al*. [68]. The influence of temperature on the dissociation of AAHP and on the stability of its metal complexes in the monomeric and polymeric forms were critically studied, on the basis of the thermodynamic functions, The dissociation process of AAHP was found to be non-spontaneous, endothermic and entropically unfavorable, whereas the complex formation were spontaneous, endothermic, and entropically favorable behavior.



Scheme (1-11) [From ref.(68)]

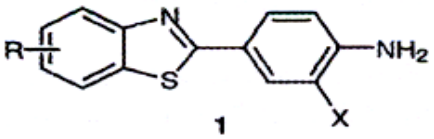
Thermoanalytical investigation of Ni(Π),Co(Π),and Cu(Π) complexes with imidazole– 4 – acetic acid(IAA) were reported by Mater and Vasca[69]. They showed that the imidazole ring and its derivatives where useful models to understand the coordination properties and the reaction mechanisms of the biologically important systems where these molecules were involved. Six different complexes of (IAA) with Co(II), Ni(II) and Cu(II) were seperated and characterized by elemental analysis, uv-vis and IR spectroscopy. The tetrahedral coordination of the Co(Π) atom includes two carboxyl groups from two different (IAA) molecules.

1.6 Importance of Azo Dyes in Biochemical Studies:

Metal complexes play an essential role in many fields. They are used extensively in analytical chemistry and of course have vital roles in biological systems, for example, the removal of copper in Wilson's disease[70].For this reason, many workers [71-77] have been concerned with

the chemistry of azo dyes derivatives, That are a attributed their role, in many biological and biochemical reactions. Azo dyes may be involved in the formation of intermediates in vivo during the metabolism process.

Some azo dyes were published as antimicrobial agents[74], for example; prontosil salicyl azo, sulfopyriden and 6-(4-hydroxy pheny azo) uracil In 1998, Bradshow *et al.* [78] showed that 2-(4-Amino phenyl) benzothiazole derivatives were a novel class of potent and selective a ntitumour agents which exhibit nanomolar inhibitory activity against a range of human breast, ovarian, colon and renal cell lines in vitro as shown in scheme (1-12) .

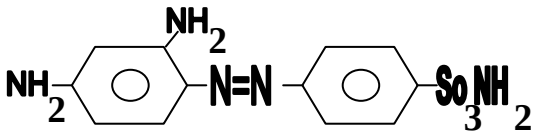


1

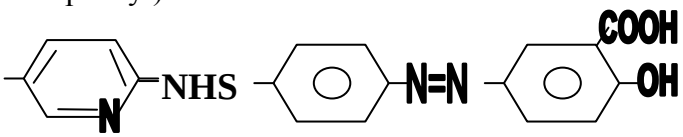
X = H,Me,Cl,Br,I

R = H,F

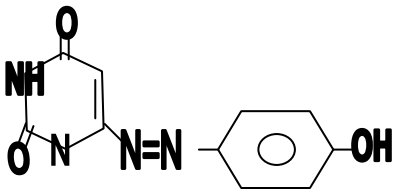
2-(4-Amino phenyl) benzothiazoles



Prontosil



Salicylazo Sulfopyriden



Uracil

Scheme (1-12) [From ref. (74), (78)]

Metero amidizoles (Flagil R) is know anti protozoal drug. Meantime, it is active against anaerobic bacteria [75] leramisal is an anti-helmenthic agent as well as nonspecific immunstimulants[76] . Likewise Pander and Tandon [77] synthesized some new quinolyl imidazoles

derivatives for their antiviral and antifungal 0activities, underlining the potential of these compounds for human medicine

1.7 Aim of the present Work:

The present investigation concerned with the study of the relative stability of some azo dyes complexes with certain metals. Attention has been directed to investigate different factors that may affect the ionization and stability constants of the prepared complex then study the compound anti microbial activity against different bacteria and fungus.

For these reasons, the following steps have been undertaken in the course of this investigation:

1. Preparation of three series of azo dyes namely Benzothiazole, Benzimidazole, and Triazole derivatives.
2. Characterization of these compounds by different techniques.
3. Studying of some physicochemical properties.
4. Determination of the ionization constants, using potentiometric method.
5. Determination of the apparent thermodynamic stability constants of

   complexes formed by reactions of these azo dyes with UO_2 (II), Ce(III), La(III), Pb(II),In(III), Zn(II), Cu(II), Ni(II) using a spectrophotometric method.
6. In order to study the properties of the bonds formed between The prepared azo dyes and metal ions, the thermodynamic functions ($\Delta H^O, \Delta G^O and \Delta S^O$) should be determined by, separation of ΔH^O into electrostatic and covalent components in order to relate these values to the type of bonding between the metal ion and the ligand.
7. Biological activity of these compounds against certain types of

bacteria were tested.

Chapter Two

Experimental:

2.1 Chemicals:

All chemicals used in this study were presented in Table (2-1) and were used directly without any further purification: distilled water was used throughout the experimental work.

Table (2-1) : Chemicals

Substance	Company	Purity
Sodium Nitrite	B.D.H	99.5%
Potassium Nitrate	Merck	98.5%
4- Nitro Aniline	B.D.H	99.0%
4-Methyl Aniline	Merck	99.5%
4-Bromo Aniline	Merck	98.5%
Bromine	BDH	98.0%
1.4 Dioxane	BDH	99.0%
Acetone	BDH	99.5%
Chloroform	BDH	98.5%
5.Amino Salicylic Acid	Flucka	97%
2.Amino Benzimidazole	BDH	98.5%
Hydrochloric Acid	BDH	12N
Sulfuric acid	Aldrich	18N
3.Amino 1,2,4 Triazole	Aldrich	99.0%
Potassium Iodide	Aldrich	98.0%
Uranyl Acetate	Aldrich	Analar

Cerium Nitrate	Aldrich	Analar
Cadmium Chloride	Aldrich	Analar
Indium Perchlorate	Aldrich	Analar
Zinc Sulphate	Aldrich	Analar
Copper Nitrate	Aldrich	Analar
Nickel Chloride	Aldrich	Analar
Lead Nitrate	Flucka	99.3%
Lanthanium Acetate	Flucka	97.2%
Amonium Aniline	Riedel – dehaen AG	96.0%
2-Mercapto Aniline	Merck	Techn.
8-Hydroxy Quinoline	Merck	98.60%
Sodium Chromate	BDH	98.6%
Dimethyl Glyoxime	Aldrich	Tech
Salicylaldoxime	Aldrich	98.5%
Amonium Phosphate	BDH	97.3%
Sodium Hydroxide	Flucka	Analar
Glacial Acetic acid	BDH	Pur.
5-Bromo Salicylic Acid	Flucka	97%
Mueller – Hiuton Ager	Oxoid	Tech
5-Methyl Salicylic Acid	Flucka	97%

2.2 Experimental Apparatus and Instrumentation:

Perspex vessels were used in all experimental work and the instruments used during this work are:

1 - Pye Unicam (sp8-100) Uv-Vis. Spectrophotometer.

2 - Pye Unicam (PU8800) Uv-Vis. Spectrophotometer.

3 - Pye Unicam (sp3-100) Infrared Spectrophotometer.

4 - Jenway 30202 pH – Meter.

5 - Stuart Melting points Apparatus.

6- GC-ITD(Ion Trap Detector)Prikin Ealmar Mass spectrometer(USA).

7- Incubator, Termaks ,USA.

8- Perkin Elmer, C.H.N Analysis.

2.3.Experimental procedures:

2.3.1 Preparation of 2- Amino 6 – Methyl Benzothiazole[79]:

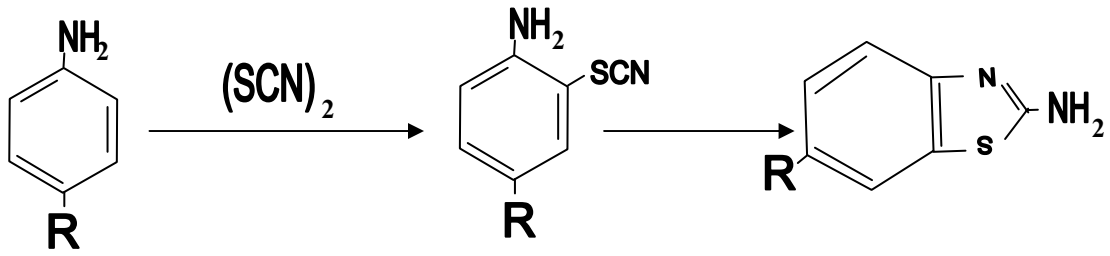
A mixture of (2.14 gm) 0.03 mole of 4 – methyl aniline, (3.65 gm) 0.034 mole of ammonium thiocyaniate and of glacial acetic acid 70 ml were placed in a flat bottomed flask surrounding by a mixture of ice and water. When the temperature falls to 10.5, a solution of 1.2 ml of bromine and with 50 ml glacial acetic acid was added slowly during 2 hour from a separating funnel, maintaining the temperature lower than $10C^{o}$, otherwise a brownish color and a heavy oil product would be formed.

The solution was poured into a beaker containing 200 ml (10C°)cold distilled water .A solution of one molar sodium hydroxide was used to precipitate the product the pH of the solution should be maintain at (9 – 10), A yellow crystals were precipitated, and a recrystallization from

ethanol was done twice before using, m.p.136^{o}C The percentage yield 62%. The procedure was seen in scheme (2-1).

Similar procedure was used to prepare 2–amino- 6-bromo and 2 – amino – 6–nitro – Benzothiozole.

$Br_2+2NH_4SCN \longrightarrow (SCN)_2+2NH_4Br$



R : CH ,NO and Br

Scheme (2-1)

2.3.2 Preparation of 2 - Hydroxy 5 – Iodo Benzoic Acid[80] *:*

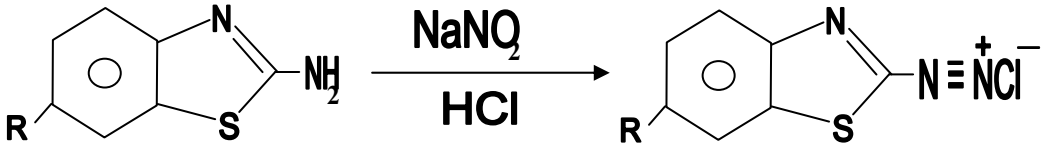
5.amino 2-hydroxy benzoic acid was (10 gm) dissolved in a mixture of (32.1) ml of concentrated sulfuric acid, 250 ml of distilled water and crushed ice in a beaker. The solution was cooled to 0 – 5C^{o} and, a solution of (4.7 gm) of sodium nitrite in (75 ml) of water was added, The mixture was stirred for 20 min. .The cold diazonium salt solution was poured into an ice – cold solution of (12 gm) of potassium iodide in 100 ml of water contained in a beaker provided with a mechanical stirrer. After 15 minutes, (1 gm) of copper bronze (which was previously washed with ether), was added and warmed slowly on a water bath, maintaining the temperature at 75 – 80C^{o} until the evolution of nitrogen gases began. The separated a

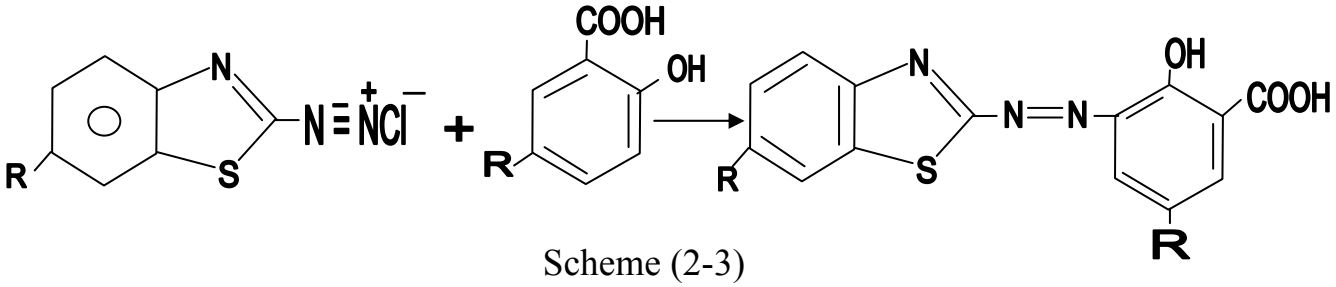
heavy oil, was extracted with chloroform; the extract was washed with dilute sodium bisulphite .[80] The 5-iodo, 2- hydroxy benzoic acid was collected, solidified on cooling and recrystallized from about 1 litter of light petroleum (b.p 80 - 100) C°. The yield was colorless product, m.p.231 – 232 C° (70% yeild), other derivative of 5- cyan 2 – hydroxy benzoic acid were synthesized by the same way described above as shown in scheme (2-2).

Scheme (2-2)

2.3.3 Preparation of Benzothiazolyle Azo Dyes[80]:

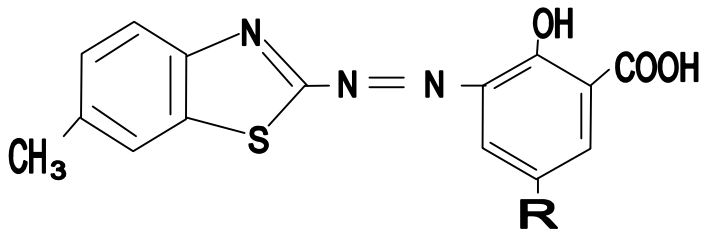
These compounds were prepared by the diazotisation of 2 – amino 6.substuent Benzothiazole and listed in Tables (2.2-2.4). The reaction took place readily in hydrochloric acid, the formed diazolate was so reactive to be coupled with phenol as shown in scheme(2.3).





Scheme (2-3)

Table (2-2) Some Physico-Chemical Data for 3-(6-Methyl – 2-Benzothiazolylazo) 2-Hydroxy Benzoic Acids Derivatives



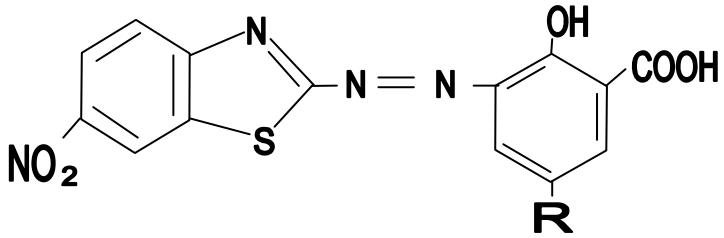
Azo dyes No.	R	M.Wt	Emp.Formula	m.p (C^o)	λ_{max} n m	Log ε l mol-1cm-1	R_f	%Yield	pK_{a1} , pK_{a2}	(CHN calc) CHN obs
1	I	439	$C_{15}H_{10}O_3N_3IS$	198-200	398	4.36 at pH=1.2	0.940	73%	2.88 , 7.70	(41.0:2.27:9.56) 40.3:2.20:9.63
2	CN	338	$C_{16}H_{10}O_3N_4S$	100-102	412	4.48 at pH=1.2	0.920	80%	3.31 , 7.53	(56.8:2.95:16.56) 56.5:2.99:15.70
3	Br	382	$C_{15}H_{10}O_3N_3BrS$	145 decomposed	404	4.29	0.922	63%	2.82 , 8.22	(47.12:2.61:10.99) 47.20:2.70:10.83
4	Cl	347.5	$C_{15}H_{10}O_3N_3ClS$	148-150	392	4.48	0.891	65%	2.96 , 8.66	(51.79:2.87:12.08) 51.90:2.91:12.20
5	CH_3	327	$C_{16}H1_{13}O_3N_3S$	142-144	420	4.68	0.963	68%	2.62 , 9.21	(58.71:3.97:12.84) 58.99:3.91:12.87

Infrared spectra analysis

For azo dyes no.5 as shown in Fig (3-1)

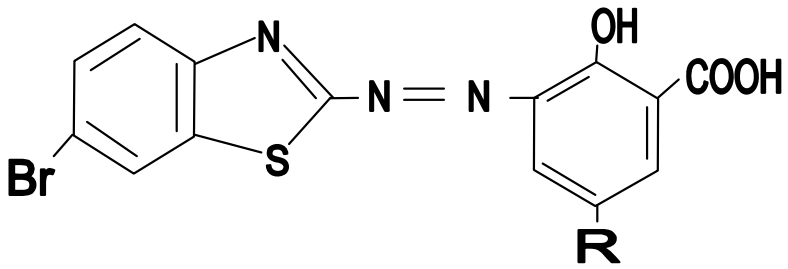
Selected IR bands	ν (-C=S)	ν (-C=N)	ν (-N=N)	ν (-C=C)	ν (-C=O)	ν (-OH)
Wave no(cm^{-1})	1220s	1410s	1460m	1575m	1675m	3200b

Table (2-3) Some Physico-Chemical Data for 3-(6-Nitro– 2-Benzothiazolylazo) 2-Hydroxy Benzoic Acids Derivatives



Azo dyes No.	R	M.Wt	Emp.Formula	m.p (C^o)	λ_{max} n m	Log ε l mol-1cm-1	R_f	%Yield	pK_{a1} , pK_{a2}	(CHN calc) CHN obs
6	I	470	$C_{14}H_7O_5\ N_4IS$	140-142	405	4.90	0.890	68%	2.50 , 7.63	(35.74:1.48:11.91) 36.01:1.50:11.80
7	CN	369	$C_{15}H_7\ O_5N_5S$	113-115	404	4.74	0.971	70%	2.40 , 7.17	(48.78:1.89:18.97) 49.07:1.91:18.81
8	CH_3	358	$C_{15}H_{10}\ O_5N_4S$	170-172	417	4.73	0.953	70%	2.99 , 9.12	(50.27:2.79:15.64) 50.51:2.80:15.72
9	Br	423	$C_{14}H_7\ O_5N_4Br\ S$	135-137	394	4.72	0.950	60%	2.30 , 8.10	(39.71:1.65:13.23) 39.80:1.70:13.38
10	Cl	378.5	$C_{14}H_7\ O_5N_4\ Cl\ S$	105-107	391	4.79	0.937	78%	2.66 , 8.75	(44.38:1.85:14:79) 44.09:1.99:14.81

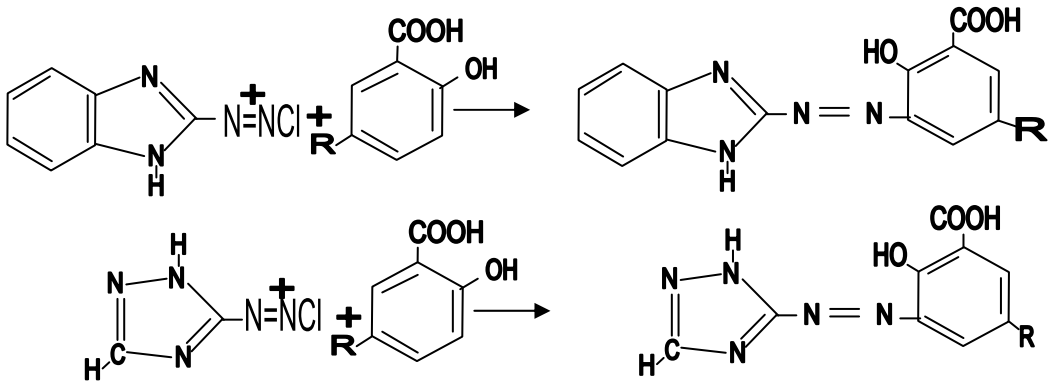
Table (2-4) Some Physico-Chemical Data for 3-(6-Bromo– 2-Benzothiazolylazo) 2-Hydroxy Benzoic Acids Derivatives



Azo dyes No.	R	M.Wt	Emp.Formula	m.p (C^o)	λ_{max} n m	log ε l mo^{l-} $1cm^{-1}$	R_f	%Yield	pK_{a1}, pK_{a2}	(CHN calc) CHN obs
11	I	504	$C_{14}H_7O_3N_3BrClS$	155-157	405	4.90	0.880	75%	2.23 , 8.02	(33.33:1.38:8.33) 33.52:1.41:8.29
12	CN	403	$C_{15}H_7O_3N_4BrS$	106-108	400	4.69	0.899	70%	1.90 , 7.32	(44.66:1.73:13.89) 44.90:1.77:13.87
13	CH_3	392	$C_{15}H_{10}O_3N_3BrS$	150-152	402	4.81	0.782	60%	2.87 , 9.02	(45.91,2.55,10.71) 46.00,2.42,10.75
14	Br	457	$C_{14}H_7O_3N_3Br_2S$	162-164	389	4.09	0.911	60%	2.68 , 7.92	(36.76:1.53:9.19) 36.90:1.60:9.23
15	Cl	412.5	$C_{14}H_7O_3N_3ClBrS$	131-133	412	4.58	0.799	65%	2.53 , 8.07	(40.72:1.69:10.18) 40.83:1.71:10.31

2.3.4: Preparation of Benzimidazolyle and 1,2,4 Triazolyle Azo Dyes [81]:

Benzimidazole azo dyes were synthesized by coupling diazotised 2-amino benzimidazole with the corresponding phenol in an ethanol at 0-5C^{o}, in similar way traizolyle azo dyes were prepared [81]. The detail experimental procedure was described previously[82].(Scheme(2-4). The compounds which were prepared in the way were reported in Tables (2-5-2-6).

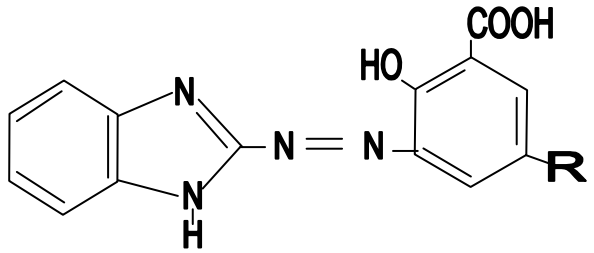


Scheme (2.4)

2.4 Purification of Azo Dyes:

No special conditions are required for the diazotisation of 2-amino-benzothiazole and its derivatives in contrary to the preparation of analogous 2 – amino-benzimidazole and 3 – amino triazole derivatives [81] , All azo dyes needed to be free from impurities such as inorganic salts, organic raw materials and water contents. Recrystallization from ethanol, dissolution of the dye in sodium hydroxide solution, followed by extraction with diethyl ether, and subsequent reprecipitation with dilute hydrochloric acid were applied to purify these compounds before they have been used in the study determination of their ionization constants and their ability of complexation with certain metals .The purity was checked by TLC technique.

Table (2-5) Some Physico-Chemical Data for 3- (2-Benzimidazolylazo) 2-Hydroxy Benzoic Acids Derivatives



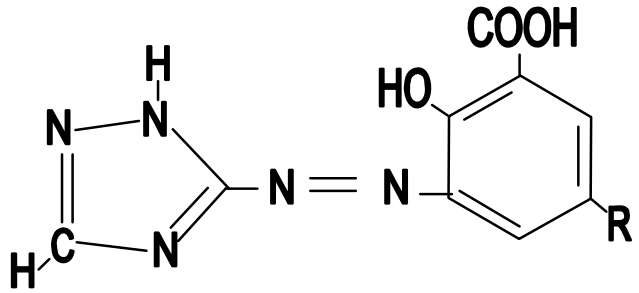
Azo dyes No.	R	Emp. Formula	M.Wt	m.p (C^o)	λ_{max} n m	Log ε l $mol^{-1}cm^{-1}$	R_f	pK_{a1} , pK_{a2}	(CHN calc) CHN obs
16	SO_3H	$C_{14}H_{10}N_4O_6S$	362	253 decomposed	404	4.052	0.953	3.63 , 8.32	(57.69:3.52:17.94) 57.73:3.48:17.68
17	Cl	$C_{14}H_9N_4O_3Cl$	316.5	293 – 295	409	3.829	0.880	3.88 , 10.82	(53.08:2.84:17.69) 53.22:2.91:17.81
18	Br	$C_{14}H_9N_4O_3Br$	361	296 decomposed	400	3.901	0.783	3.75 , 10.09	(46.53:2.49:15.51) 46.81:2.39:15.29
19	NO_2	$C_{14}H_9N_5O_5$	327	202 - 203	390	4.782	0.690	2.83 , 7.21	(51.38:2.77:21.40) 50.80:2.68:21.83

Infrared spectra analysis

For azo dyes no.17 as shown in Fig (3-1)

Selected IR bands	ν (-C=N)	ν (-N=N)	ν (-C=C)	ν (-C=O)	ν (-OH)
Wave no(cm^{-1})	1400s	1430m	1580m	1650m	3200b

Table (2-6) Some Physico-Chemical Data for (3-Triazlylazo) 2-Hydroxy Benzoic Acids Derivatives



Azo dyes No.	R	Emp. Formula	M.Wt	m.p (C^o)	λ_{max} n m	log $\mathcal{E}$ l $mol^{-1}cm^{-1}$	R_f	%Yield	pK_{a1} , PK_{a2}	(CHN calc) CHN obs
20	NO_2	$C_9H_6O_5N_6$	278	241 –242	400	4.321	0.801	80%	2.90 , 7.35	(38.70:2.5:30.1) 38.66:2.38:30.09
21	Br	$C_9H_6O_3N_5Br$	312	192 decomposed	410	4.220	0.714	70%	3.32 , 8.92	(34.61:1.92:22.93) 35.00:1.83:22.90
22	Cl	$C_9H_6O_3N_5Cl$	267.5	171 – 172	405	4.210	0.820	73%	3.21 , 10.02	(40.37:2.24:26.16) 40.58:2.40:26.50
23	$-OCH_3$	$C_{10}H_9O_4N_5$	263	191 - 192	490	3.995	0.754	65%	3.55 , 11.86	(45.62:3.42:29.33) 46.00:3.62:29.08

Infrared spectra analysis

For azo dyes no.21 as shown in Fig (3-2)

Selected IR bands	ν (-C=N)	ν (-N=N)	ν (-C=C)	ν (-C=O)	ν (-OH)
Wave no(cm^{-1})	1390m	1480s	1580s	1700m	1380b

2.5 Stock Solutions:

In order to prepare a stock ligand solution, a precise amount of azo dyes was weighted and dissolved in a known volume of 30% (v/v) dioxane – water mixed solvents, because the compounds are poorly soluble in water.

0.1M of stock metal solutions of UO_2 (II),Pb (II) Ce(III), La(III), In(III), Zn(II), Cu(II),and Ni(II) were prepared by dissolving exact analar grade salt in 30% (v/v) 1.4 dioxane – water mixed solvents, using boro silicate flasks. The solutions were arranged to contain excess acid to avoid the hydrolysis of aqua cations . The hydrolysis constants pk_{OH} of the metal ions were listed Tables (2.8-2.10) in addition to the references recommended. The solutions were analyzed to check the concentration of metal ions as follows :

Metal Ion	The Determination Method[from ref. (83,84)]
UO_2 (II)	Spectrophotometrically as thiocyanate
Pb (II)	Gravimetrically as chromate
Ce(III)	Spectrophotometrically as 2-mercapto aniline
La(III)	Gravimrtrically by EDTA
In(III)	Spectrophotometrically as 8-hydroxy quinoline
Zn(II)	Gravimetrically as the ammonium phosphate
Cu(II)	Gravimetrically by Salicylaldoxime
Ni(II)	Gravimetrically by dimethylgiyoxime

Metal analysis agreed to within ± 0.1%

2.6 Physico – Chemical Studies:

The azo dyes synthesized in this work were identified by means of elemental analysis Infra red, Uv – Vis. Spectroscopy and Mass spectra. The data are reported in the Tables (2.2-2.6).

2.6.1 Appearance:

All the azo dyes are micro crystalline powder, red to brownish in color, whose changes are expected for chromophiric groups. Mostly these compounds are springy soluble in water but soluble in organic solvents.

2.6.2 Melting Point:

The compounds have nearly sharp melting points whereas others decompose before melting .

The purity of compounds were investigated by TLC method. Rates of flow *(R_f)* were determined on silica gel TLC plates, using (n – butanol : acetic acid : water) with ratio 4 : 1 : 5 respectively by volume as eluent the rate of flow values are listed in the Tables (2.2-2.6) .

2.6.3 Uv – Vis. Spectra _ :

After dissolving a precise amount of azo dyes(3×10^{-4} molar) in a known volume of mixed solvents (dioxane – water) 30% v/v 1cm quartz cell was filled. Therefore, λ_{max} was pointed and the molar extinction coefficients were obtained ; the data were listed in the Tables (2.2-2.6).

2.6.4 Infra red Spectra :

IR spectral study served as a Simple tool in understanding the molecular structure and bonding of chemical compounds in The solid state. The vibration spectra were recorded as KBr pellets in the 4000-400 cm^{-1}range. The absorption bands and their assignments were reported in the Tables (2.2-2.6) and some of the spectra are shown in Figs. (3.1-3.2) . IR spectrum for each indicated the following absorption bands: 3400 cm^{-1},1420 cm^{-1}(s), 1600 cm^{-1}(b) corresponding to the stretching frequencies for the phenolic group, azo group and aromatic structure respectively.

2.6.5 Mass Spectra :

Only four compounds namely BTZ no.2, BTZ no.7, BTZ no.10, BTZ no.17 and BTZ no.23 were subjected to mass spectra analysis. The successive fragmentation peaks are shown in Fig.(3.3-3.6). whereas (m/z) for the pyrolysis within the temperature range varies between 120 – 400 C^{o} are report in Table (3.1), the details mass spectra show a peak at m/z 338, 379, 317 and 263 respectively. Exact mass measurements of these peaks reveal the presence of the azo dyes under consideration.

$$K_1^H = \frac{[LH]}{[L][H]} = \frac{1}{K_{a.1}}$$

$$K_2^H = \frac{[LH_2]}{[LH][H]} = \frac{1}{K_{a.1}}$$

In general:

$$K_n^H = \frac{[LH_n]}{[LH_{n-1}][H]}$$

The over all association content is given by

$$B_n^H = K_1^H . K_2^H ..... K_n^H$$

$\mathbf{K}_a$ is usually small and an inconvenient figure and hence it has become customary to use their negative logarithms (known as pK_a values). The activity coefficients for all species are kept constant by using a non reacting background electrolyte of high concentration relative to that of the reaction species.

Ionization equilibria for protonated azo dyes BTZ, BIZ, and TZ azo phenols can be written as

$$H_3L^+ \overset{pK^-_a\,(N^+H)}{\rightleftharpoons} H_2L \overset{pK_{a1}COOH}{\rightleftharpoons} HL^- \overset{pK_{a2}\,(O.OH)}{\rightleftharpoons} L^{2-} \quad ....(2.3)$$

The degree of formation of protonated ligand species $\bar{n}$ is

$$\bar{n}_H = \frac{\text{Number of hydrogen ions bound to ligand} \quad (C_H)}{\text{Total number of ligand molecules} \quad (C_L)} \quad ...(2.4)$$

C_H =total hydronium ion concentration=[H]+[LH]+2[LH_2]+…..+N [LH_n]

$$= [H]+B_1^H[L][H] + 2B_2^H[L][H]^2 + ... + nB_n^H[L][H^n]$$

$$= [H]+[L]\sum_1^N nB_n^H [H]^n$$

CL= total ligand concentration =[L]+[LH]+[LH_2] +…….+[LH_n]

$$= [L]+B_1^H[L]H + B_2^H[L][H]^2 + ... + B_n^H[L][H]^n$$

$$= [L]\sum_0^n B_n^H [H]^n$$

$$\bar{n}_H = \frac{\sum_1^N nB_n^H [H]^n}{\sum_0^N B_n^H [H]^n}$$

N=2 for the ligands under investigation, thus: -

$$\bar{n}_H = \frac{B_1^H[H] + 2B_2^H[H]^2}{1 + B_1^H[H] + B_2^H[H]^2}$$

$$\bar{n}_H = \frac{[H]/K_{a2} + 2[H]^2/K_{a1}K_{a2}}{1 + [H]/K_{a2} + [H]^2/K_{a1}K_{a2}}$$

$$\bar{n}_H + \bar{n}_H[H]/K_{a2} + \bar{n}_H[H]^2/K_{a1}K_{a2} = [H]/K_{a2} + 2[H]^2/K_{a1}K_{a2}$$

$$\bar{n}_H[H] + \bar{n}_H/K_{a2} + \bar{n}_H[H]/K_{a1}K_{a2} = 1/K_{a2} + 2[H]/K_{a1}K_{a2}$$

$$K_{a1} = \frac{[H](2-\bar{n}_H)}{(\bar{n}_H^{-1}) + (\bar{n}_H K_{a2}/[H])}$$

And $$K_{a2} = \frac{[H](1-\bar{n}_H) + (2-\bar{n}_H)[H]/K_{a1}}{\bar{n}_H}$$

If the constants are sufficiently different (more than 3 log units in pK) so: -

$$PK_{a1} = PH + \log \frac{(\bar{n}_H - 1)}{(2-\bar{n}_H)} \quad - \ \mathbf{1 < n_H < 2}$$

$$PK_{a2} = PH + \log \frac{\bar{n}_H}{(1-\bar{n}_H)} \quad \textbf{Where} \quad - \ \mathbf{0 < n_H < 1}$$

$$\bar{n}_H = \frac{C_H - [H] + K_w[H]^{-1}}{C_L}$$

$$0.2 \le \bar{n}_H \le 0.8$$

2.7.2 Experimental details:

The potentiometric method was used for the determination of ionization constants .The titration was conveniently carried out in a beaker which was closed by a cork with four holes, for the combined electrode, nitrogen inlet, thermometer and one admits the tip of burette. Stirring accomplished by slow stream of nitrogen bubbles was introduced under the surface of solution to be titrated. This flow should be stopped during readings [87]. The titrant (sodium hydroxide solution) which was used would

be in ten portions concentration than that azo dye solution in flask, to avoid the dilution effect during the titration process and the pH reading. The pH value was recorded when the equilibrium was attained after each addition. The ionic strength was kept constant with 0.1 M potassium nitrate. Gauss computer program [88] was used to achieve the purpose of finding the ionization constants.

The of pK_{a1} and pK_{a2} values of BTZ, BIZ and TZ azo dyes were reported in Tables (2.2-2.6). In the complex formation studies, it is convenient to describe the system containing ligand molecules and hydrogen ions in terms of association constant rather than ionization constant.

2.8 Determination of Thermodynamic Stability Constants:

2.8.1 Theoretical Aspect:

The qualitative role in the determination of the number complexes formed in the solution spectrophotometrically was described by Beck [12]. A continuous variation method and mole ratio method were used revealing that (1:1) metal. To Ligand chelates were formed in these experimental conditions in acidic medium as indicated in Table(2-7-2.9).

If one complex is formed, one can apply the method of the spectrophotometric determination of the stability constants developed by many workers [89-91]. This method is only applied when the metal and ligand react are one constant ratio.

2.6 Ionic BackGround:

In order to control activity coefficient in aqueous solutions by the use of constant ionic background, an excess of an electrolyte was used, which is usually assumed not to form any complexes with the ligands or metal ions. The electrolyte might be soluble to give an ionic medium with the appropriate concentration. In this work, an ionic background of 0.1M of nitrate used. Sillen and Bieder [85] suggested that for a system , in which the free cation concentration was varied.

2.7 Determination of Acid Dissociation Constants of the Protonated Azo Dye:

2.7.1 Theoretical Aspects:

Ionization constants are a function of acid and base strengths some times referred to as dissociation constants, which reveal the properties of the different ionic species into which a substance is divided at any chosen pH [86].

For acid, the ionization process is

$$LH \rightleftharpoons H^{+} + LH_{n-1} \qquad \text{.....(2.1)}$$

The stocheiometric acid ionization constant is given by

$$K_{an} = \frac{[LH_{n-1}][H]}{[LH_{n}]}$$

It is convenient to express the system confining so ligand molecules and hydrogen ions terms of association constants (K_n^H) rather than ionization constant (K_{an})

H_2L represents the azo dye with the following equilibria:

$$H_2L \overset{Ka1}{\rightleftharpoons} HL^- + H^+$$

$$HL \overset{Ka2}{\rightleftharpoons} L^{-2} + H^+$$

When the reaction occurs between metal ion(M^{n+}) and azo dye solution, the expected equilibria are:-

$$HL^- + M^{n+} \rightleftharpoons ML^{(n-2)+} + H^+$$

$$L^{2-} + M^{n+} \rightleftharpoons ML^{(n-2)+}$$

The above equilibria can be described in terms of thermodynamic equilibrium constant

$$K_{a1} = H\,[HL] / [H_2L] \quad \ldots.(2.8)$$

$$K_{a2} = H\,[L^-] / [HL] \quad \ldots.(2.9)$$

$$K_{ML} = H\,[ML] / [M][HL] \quad \ldots(2.10)$$

$$\text{So } K_a = K_{a1}.\, K_{a2} = \frac{[H]^2\,[L]}{H_2L} \quad \ldots..(2.10)$$

$$\text{And} \quad K_{ML} = \frac{[H^+][ML]}{[M][HL]} \quad \ldots(2.11)$$

Charges are omitted for the sake of simplicity, where the total concentration of the ligand C_L in the solution is:

$$C_L = [H_2L] + [HL] + [L] + [ML]$$

The total concentration of the metal C_M is:

$$C_M = [ML] + [M]$$

where a metal ion reacts with H_2L, at relatively low pH (to avoid the hydrolysis phenomena of the metal ion) .

L^{2-},[M $(OH)_y$] and, [MHL]are negligible in the pH range (pH=3.3-4), whereas the ionic strength is still constant (0.1μ KNO_3), The hydrolysis constants pK_{OH} of the metal ions were listed in Tables (2.7-2.9) in addition to the recommended references. In the presence a large excess of C_M over that of the C_L, the type of complex formed at that condition are found by using continuous variations method (job's method)[12 , 92], or. and the mole ratio method [93.94]. absorbance was measured wave length of miximinum absorption(max), where only the chelate was absorbed , indicated that (1:1) metal: ligand was formed under that condition so $\varepsilon_M = \varepsilon_{HL} = \varepsilon_{H2L} = 0$. And the following empirical equation was used:

$$\frac{C_M}{A} = \frac{1}{\varepsilon_{ML}.l} + \frac{1}{\varepsilon_{ML} l K_{ML}} \left[\left(H(Hf_2^2 + K_{a2} \right) / f_1 (C_L - \frac{A}{\varepsilon_{ML}.l}) \right] \quad \ldots(2\text{-}12)$$

where, A is the absorbance at a fixed wavelength **ε** the molar absorptirity coefficient of the ligand, L and is the bath length of the quartz cell .

The variable (Y= C_M / A) ranged from 1 / ε l to 3 / εl in such a way as to maximize the accuracy in the slope and the intercept values. The stability constants were calculated, following the least square method, using (C_M) the total concentration of ML, the ligand, the free hydronium ion concentration (H) and the absorbance of the complex (A).

2.8.2 Experimental Detail of determination of Stability Constants:

To determine the stability constant for complex formation, standard solutions of freshly prepared azo dyes and metal ions of ($1X10^{-5}$ and$4X10^{-4}$ M) in mixed solvents (30% dioxane-water by volumes) are mixed. A series of test solutions were prepared and the electronic absorption spectra were recorded by the following steps:

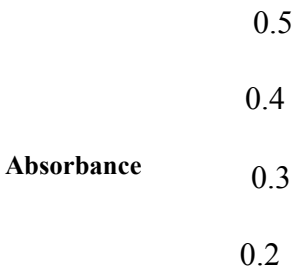
1- The Uv-Vis spectrum of the azo dye and the metal ion were recorded individually, in 30 % ,1.4 dioxane/water.

2- The wave length of maximum absorption (λ_{max}) of the azo dye and its complex with the metal ion (for each) were found.

3- The absorbance of the complexes formed at(λ_{max})and $25C^{o.}$ were found listed in the Tables (2-7 – 2-9).

4- In order to study the effect of temperature on the stability constants, the absorbance of the complex solutions should be determined at different temperatures (18, 25, $32C^{o}$). The required temperature for each reaction was obtained and maintained to about (0.1-0.2 C^{o}) in the cell compartment by circulating water thermostat through the cell compartment for about 15 min. to initiate the reaction. Taking in consideration the fact that the reagents used for complex formation were kept at constant temperature (in the water bath). The absorbance of the complex solution was recorded against the blank, (which contained all the reagents except the metal ion).

5- The pH-values of the solutions were needed to calculate the stability constants by applying equation. (2-12)


0.5
0.4
Absorbance
0.3
0.2

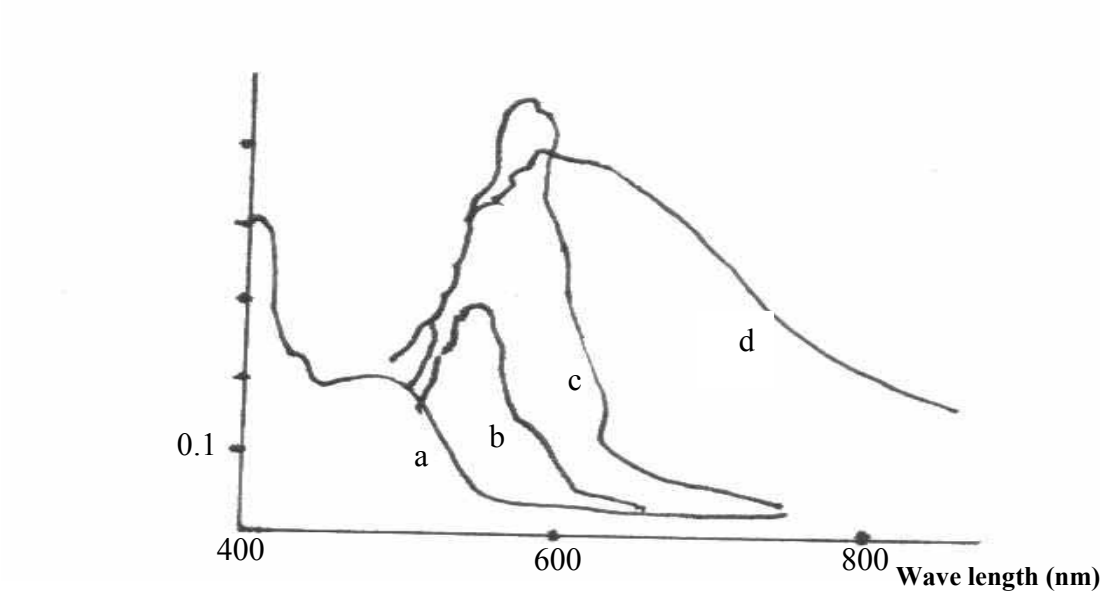


Fig (2-1) Visible spectra of BTZ azo dye no.7 (a) and its complexes with (b) Zn (II), (c) Pb (II) and (d) UO_2 (II) ions

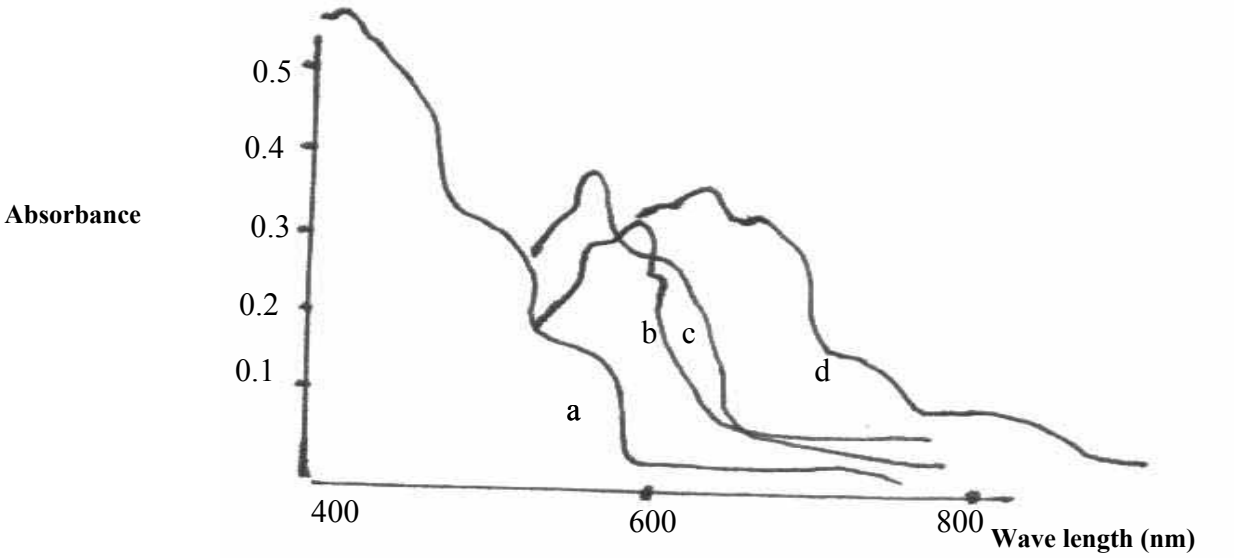


Fig (2-2) Visible spectra of BIZ azo dye no.17 (a) and its complexes with (b) UO_2 (II), (c) In (III) and (d) Cu (II) ions

Table (2-7) Characterization Data for The Complexes of BTZ azodyes (N0. 1-15) with the Metal ions indicated

Metal Ion	pk_{OH} Ref(96-103)	λ_{max} nm	$\varepsilon . 10^3$	pH range	Color of the Complex	Log K_{ML}
UO_2 (II)	9.3	580-600	19-20	3.7-4.0	r-o	7.7-9.0
Pb (II)	6.5	575-600	19-20	3.8-4.3	y-p	7.2-8.5
Ce (II)	4.3	560-630	18-20	3.2-4.1	y	8.5-9.5
La (III)	8.3	560-620	17-18	4.0-4.3	y-o	6.0-7.2
In (III)	4.4	620-695	21-23	3.0-3.8	bl.	10.0-9.0
Zn (II)	8.2	590-620	16-18	4.0-4.7	o-y	6.5-7.5
Cu (II)	6.8	570-640	22-23	3.9-4.7	p-r	10.0-11.2
Ni (II)	6.5	550-625	18-19	3.8-4.4	y-o	7.5-8.3

Table (2-8) Characterization Data for the Complexes of BIZ azo dyes (No. 16-19) with the Metal ions indicated

Metal Ion	pk_{OH}	λ_{max} nm	$\varepsilon . 10^3$	pH range	Color of the Complex.	Log K_{ML}
UO_2 (II)	9.3	580-600	16-18	4.0-4.5	wine-r	8.6-9.4
Pb (II)	6.5	590-610	18-20	4.2-4.6	y-p	8.5-9
Ce (III)	4.3	570-585	18-21	3.0-4.0	y-o	10.7-11.5
La (III)	8.3	590-620	18-19	4.0-4.5	y	8.3-9.3
In (III)	4.4	600-630	20-23	3.0-4.0	bl-g	11.3-12.2
Zn (II)	8.2	500-620	15-15.5	4.0-5.0	wine-r	6.5-7.3
Cu (II)	6.8	570-620	22-34	7.5-4.5	Br-p	8.8-9.2
Ni (II)	6.5	570-630	17-19	4.0-5.0	y-p	7.7-8.0

Table (2-9) Characterization Data for The Complexes of TZ azo dyes (No. 20-23) with the Metal ions indicated

Metal Ion	pk_{OH}	λ_{max} nm	$\mathcal{E} . 10^3$	pH range	Color of the Complex.	Log K_{ML}
UO_2 (II)	**9.3**	**550-670**	**9-10**	**4.0-4.9**	**o-r**	**4.2-5.3**
Pb (II)	**6.5**	**500-650**	**8-9**	**4.3-5.2**	**Y**	**3.0-3.8**
Ce (III)	**4.3**	**520-660**	**11-13**	**3.2-4.1**	**y-r**	**6.3-7.2**
La (III)	**8.3**	**500-620**	**11-13**	**4.5-5.0**	**y-o**	**4.7-5.0**
In (III)	**4.4**	**505-630**	**9-10**	**3.3-4.0**	**y**	**5.3-6.2**
Zn (II)	**8.2**	**590-630**	**8-9**	**3.2-4.0**	**r.**	**3.2-3.7**
Cu (II)	**6.8**	**520-660**	**11-13**	**4.0-4.7**	**br.**	**6.0-7.4**
Ni (II)	**6.5**	**570-605**	**12-13**	**4.5-5.5**	**P**	**5.5-6.3**

*bl: blue , g: green, br: browen , y: yellow , r: red and p: purple

2.9 Determination of Thermodynamic Functions for Complex Formation:

Vant' Hoff deduced a quantitative relationship between the changes in equilibrium constants and the variation in the temperature [104]. (ΔH^O) can be obtained from the well known equation

$$\ln K_{ML} = -\Delta H^O / RT + \text{constant} \quad \text{.... (2.14)}$$

where R represents gas constant (8.3143 jK^{-1}), and T the temperature in Kalvin In this work, the stability constants were obtained pectrophotometrically at three different temperatures (18, 25, 32) ^oC . So (ΔH^O) could be estimated using least squares method. For a dilute solution, ΔG^O and ΔS^O could be calculated by the following equation:

$$\Delta G^O = -RT \ln K_{ML} \quad \text{....(2.15)}$$

$$\Delta S^O = (\Delta G^O - \Delta H^O)/T \quad \text{....(2.16)}$$

For the aim of detailed discussion of thermodynamic quantities, it is convenient to analyze the changes in enthalpy in terms of (ΔH_{el}) electrostatic enthalpy change represented long range of contribution depended upon the environmental and temperature changes. The covalent component, (ΔH_c) the cratic enthalpy change, represents a short range of contribution independing upon temperature change and environments

$$\Delta H = \Delta H_{el} + \Delta H_c \quad \text{....(2.17)}$$

The separation of thermodynamic functions into temperature dependant and temperature independent components was firstly suggested by Gurney [105] for proton ionization reactions. The method was extended to

metal complex formation by Nancollas [106] and recently also been applied by Mashaly et al . [59] .

So ΔH_{el} was obtained using the equation below:

$$\Delta H_{el} = (T - r)(\Delta S + \Delta nR \ln x) \quad \ldots.(2.18)$$

Where T represent the temperature of the solution during the reaction process between metal ion and ligand reach the equilibrium. (r): characteristic temperature of the solvent(for water = 216 Kelvin).

Δn the change of the solute particles during the complexation process, and (x) the number of water molecules per 1000 gm. of solvent.

$$\Delta H = \Delta H_{el} + \Delta H_{c}$$

This equation was applied in the work to calculate (ΔH_c) and (ΔH_{el}), data are reported in the Tables (3-8--3-15) for the complexes under consideration.

2.10 Biological Activities:

Chemicals on facing the biological system may express multiple effects like, anti bacterial, antifungal, antiviral as well as anti neaplastic effects Besides, they may be inert [75] In the present work a preliminary study on the invitro anti bacterial effect was made for these azo dyes derivatives using disk diffusion technique

Disk diffusion technique: diffusion method was used to make preliminary investigation of anti bacterial activity of these azo dyes derivatives invitro, the method ,depends on the diffusion of a known concentration of the tested agents from a filter paper disk applied on a seeded solid surface media. The diameter of the radius of the inhibitory zone reflects the potency of the tested compound and is subjected to many physical and

chemical factors[109], such as pH, components of the medium, stability of the compound, the method size of inoculum, incubation time and metabolic activity of the microorganism.

The disk diffusion method was applied in the work to measure the bacterial sensitivity of *E.coli, Staph. aureus* and *Ps. aergenosa* to BTZ, BIZ, TZ and their complexes with Zn(II), the procedure possessed was abstracted in the following steps:

1. Mueller – Hinton agar is prepared from a dehydrated base according to the manufacturer's recommendation. The media should be capable of producing control zone sizes within the published limit NCCLS [74].
2. The medium is cooled to (45-50C^{o}) and poured into 9cm plates to a depth of 4mm set on a level surface for solidifying each plate required about 15 ml of medium.
3. When the medium is hardened, the plates for immediate use should be dried for about 30 min. at 35 C^{o} by placing them in the upright position in the incubator with the lids tilted.
4. A filter paper disk (Watman No.1) impregnating with a measured quantity of agent (about 15µg in a disk) is placed carefully on a solid surface that has been evenly seeded with test organisms (*E.coli, Staph. aureus* and *Ps. aergenosa*), using a single disk for each synthesized compound with careful standardization of test condition. The evolution of susceptibility of the microorganisms is done by measuring the diameter of inhibition zone around the disk compared with controls (disks impregnated with solvent only).
5. The size of the inhibition zones were measured to the nearest millimeter and compared with the standard limits of sensitivity of the same species of bacteria against antibiotics .

Chapter Three

Results and Discussion:-

3.1 Preparation and Characterization of Azo Dyes: -

The present work concerned with the preparation and identification of three series of azo dyes, normally. Fifteen ligands were present from the reaction of 2-amino 6-substituent benzothiozoles with 2-hydroxy 5-substituent benzoic acid, four ligands derived from 2-amino benzimidazole and the four ligands from 3-amino triazole. These ligand were identified by CHN analysis, IR-spectra technique fig (3.1 – 3.2), and only four of them were analyzed by mass spectra fig(3.3 – 3.6 and Table 3-1), in addition to the melting point and paper chromatography techniques. We would expect to obtain new highly sensitive reagents for metal ions by introducing a group which is strongly electron donating in the para position next to the azo group and or para substituant next to the hydroxy group [111], as shown in tables (2-2 – 2-6).

It has been demonstrated from Uv – vis spectra of these ligands in 30% (v/v) dioxane – water mixed solvents that the λ_{max} of the azo dyes, owing to $\pi \rightarrow \pi^*$ transition, were the region near 450 ± 50nm , the shift of the band toward lower wavelength (red shift) occurred as the ability of oxochromopheric groups to donate electrons increase through the conjugation system and vise reverse as shown clearly in tables (2.2 – 2.6).

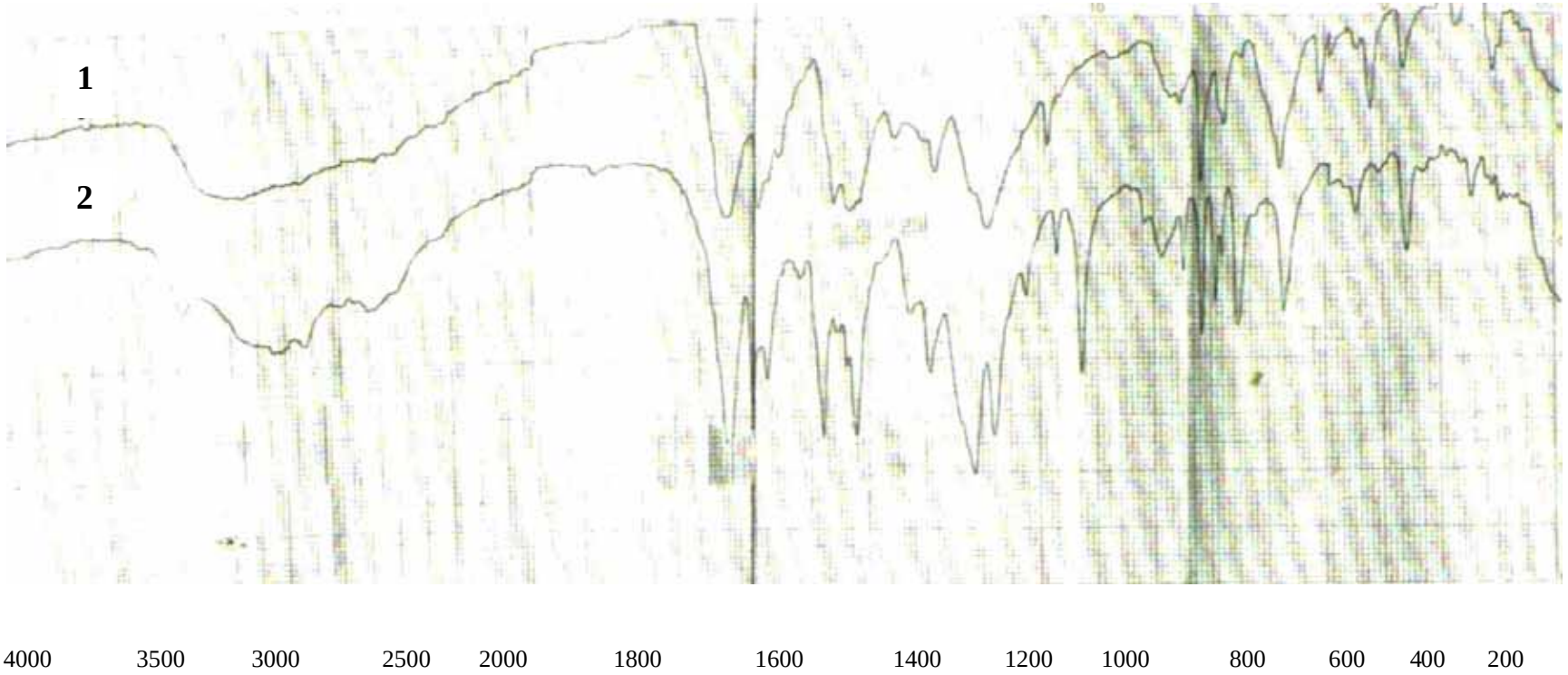
Mass spectra of only four azo dyes were performed gave a precise molecular weight for each compound under consideration as represented in the Table(3.1) addition to IR technique which assigned the presence of the

azo group in addition to clear structures of the ligands in this work as shown in the Tables(2.2-2.6).

3.2 Ionization Constants: -

The azo dyes BTZ, BIZ and TZ azo dyes have two dissociated protons in the enolized hydrogen ion of the hydroxyl group and the carboxylate of the benzoic acid, A potentiometric method was followed and Gauss computational program was used to evaluate the dissociation constants [(86)], and the data are summarized in the Tables (3-2 & 3-4), indicating that the values of the acidic character of BTZ were found to be higher these that of BIZ values, where the TZ derivatives had equivalent (pK_a) values to BTZ character.

One of the aims of the present investigation is to find out the role of each substituant, resonance effect, inductive effect and steric hindrance. According to the electron donating ability of the substituent on the pk_a values of the azo dyes following the change in the stability constants, i.e these effects are responsible for altering the reactivity of the reaction with metal ions. In order to evaluate the stability constant and a comparison was done between the three types of azo dyes (BTZ, BIZ & TZ). In general, the pK_a values assign that resonance and inductive factors play a detectable source on the pK_a whereas no steric hindrance between hydroxyl or acetate group with the substituent had been found. For a number of constituents, the obtained thermodynamic ionization constants of 23 azo dyes that were prepared , the results indicated that iodo, bromo, chloro and nitro substituted ligands have lower ionization constants compared with the methyl substituted one. one can interpret in view of electron donation, which can lead to a consequent increase the basicity in addition to entropy



Fig(3-1)Infrared absorption spectra of BIZ azo dye no.17 ;spectrum (1) and BTZ azo dye no.5 ;spectrum (2)

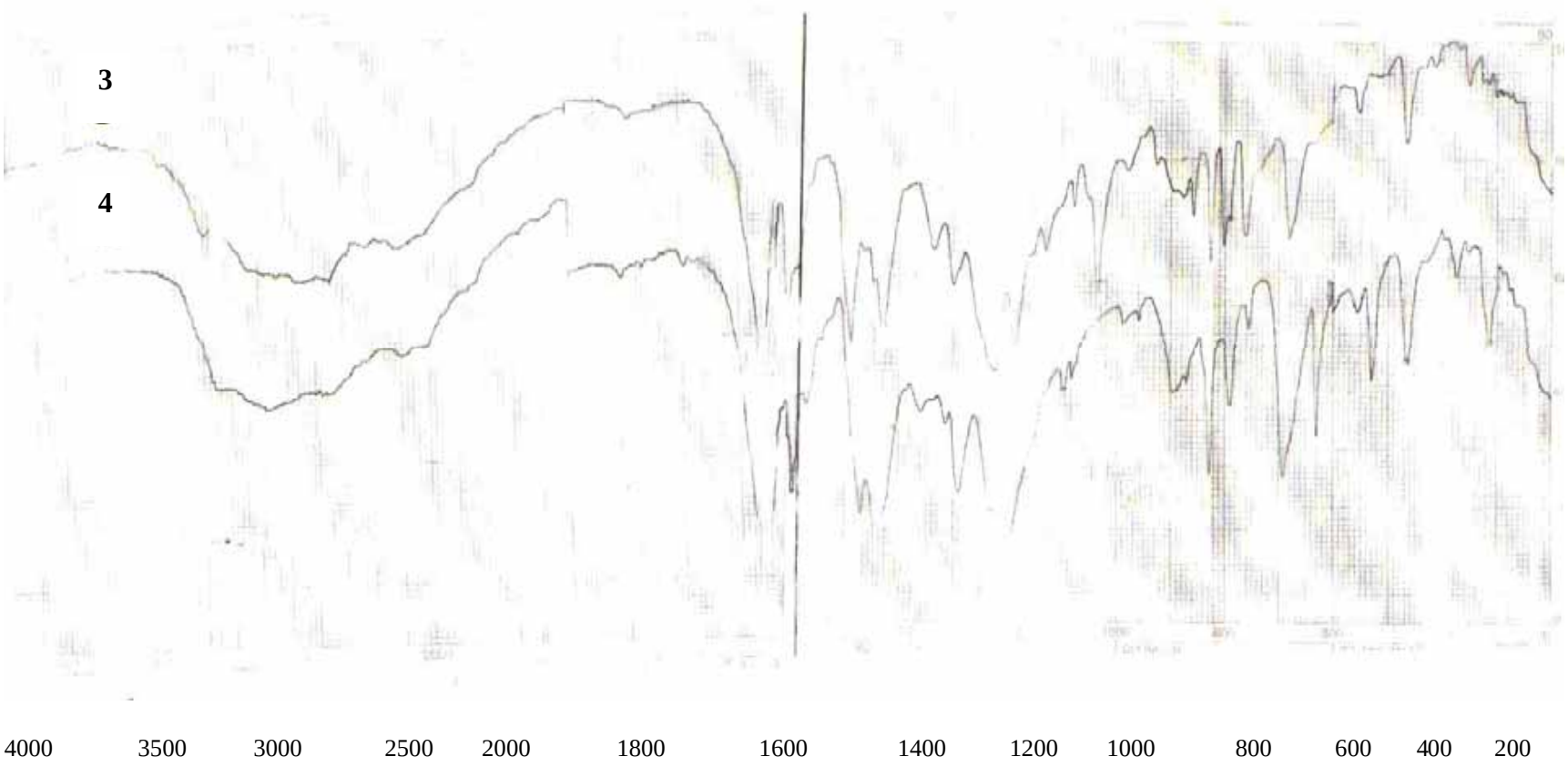
cm^{-1}

effect [112]. It is through that effect of halogen groups involving mainly the electronic effect, representing that these groups are strong electron attraction groups, through mesmeric effect, to a great extent. The stability of the proton complexes formed depends on the basicity of the hydroxyl, azo dyes under and, the availability of the electrons around the donating groups.

The pKa value of imino hydrogen equals to 14.5 in imidazole [113] . Whereas in the benzimidazole; it presumably decreases due to conjugation. However, during the work, the titration of benzimidazole up to pH (11) with sodium hydroxide no release of imino hydrogen ions observed. It was likely therefore that the pK_a of imino group is not less than 12 and not greater than 14 in the Benzimidazole derivatives, according to our investigations it was confirmed that the pK_a values increase with the increase of the mole fraction of dioxane [34].

The pK_a values indicated that the substituted 2- hydroxy benzoic acid were characterized by the absence of steric factor, assigned and that the substituents effects in the benzoic acid ring were more affective than there in the heterocyclic benzothiozolyl ring. The pK_a of BIZ is higher than of BTZ, and this might be due to the electron donating effects of the imidazole nitrogen group than in the BIZ ligands. One might expect the affinity for metal ions, rather higher stability for BIZ complexes compared with BTZ which assumed to bind through its pyridyl nitrogen and not with imidazole nitrogen.

The most probable explanation for this behavior is the presence of intramolecular hydrogen bonding[114] between the hydrogen of the o-hydroxyl group and the basic nitrogen atom of the BTZ, BIZ and TZ part of



Fig(3-2)Infrared absorption spectra of TZ azo dye no.21 ;spectrum (3) and TZ azo dye no.23 ;spectrum (4)

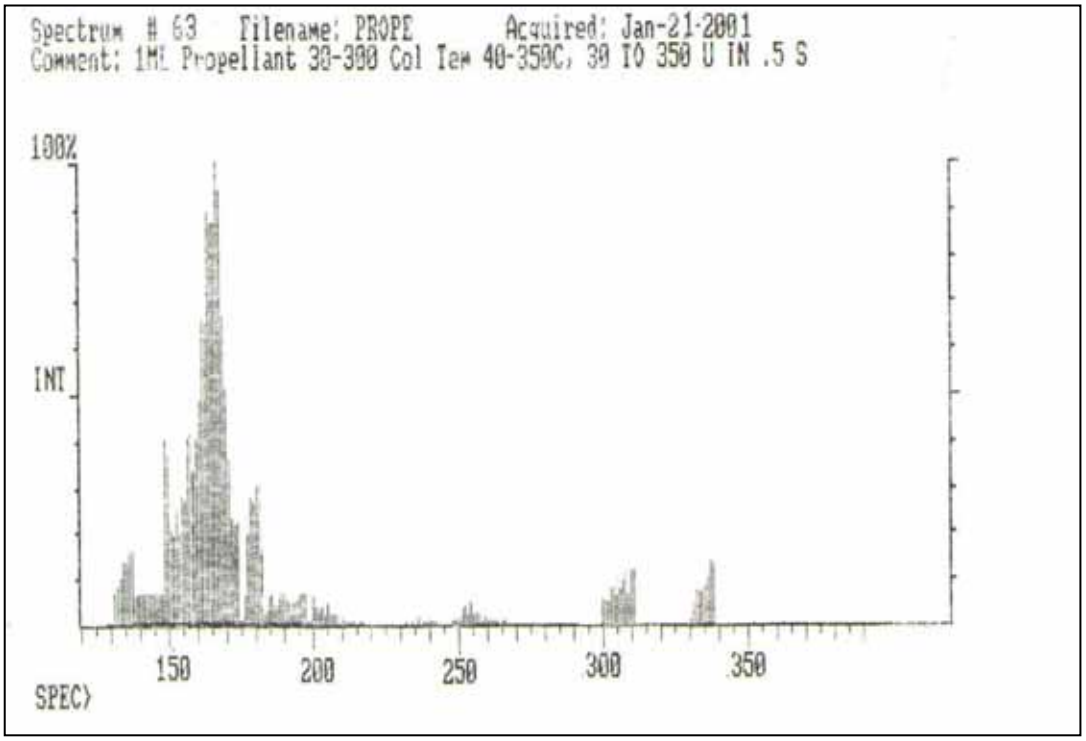


Fig (3-3) Mass spectrum of BTZ azo dye no.2

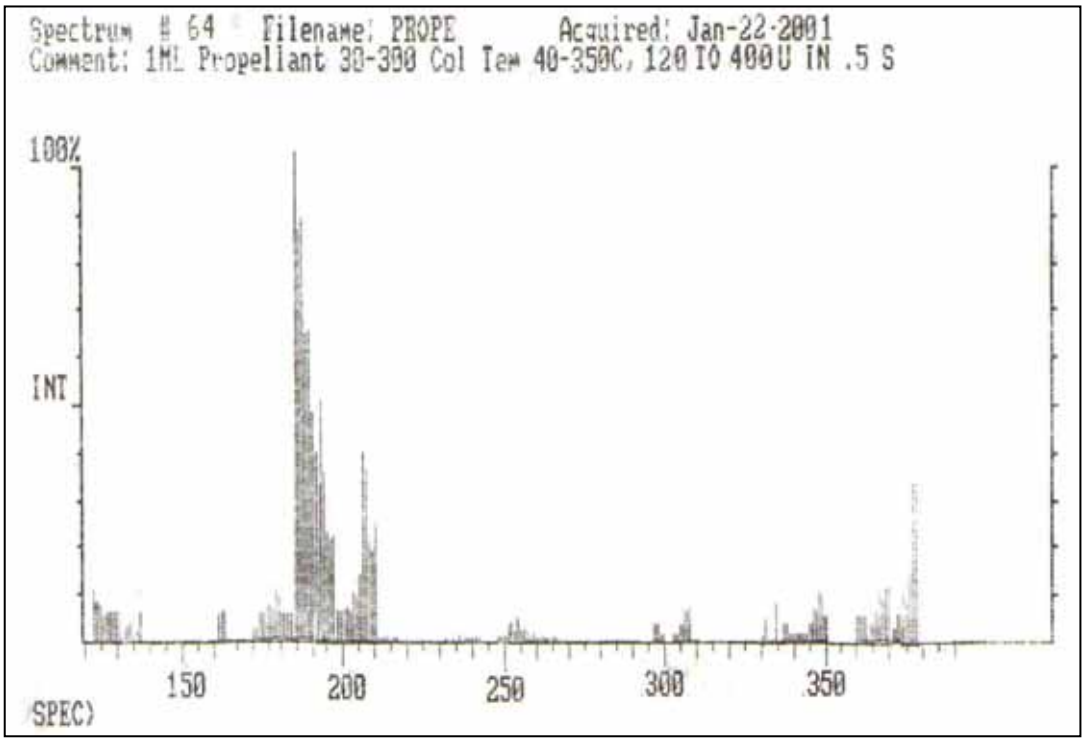


Fig (3-4) Mass spectrum of BTZ azo dye no10

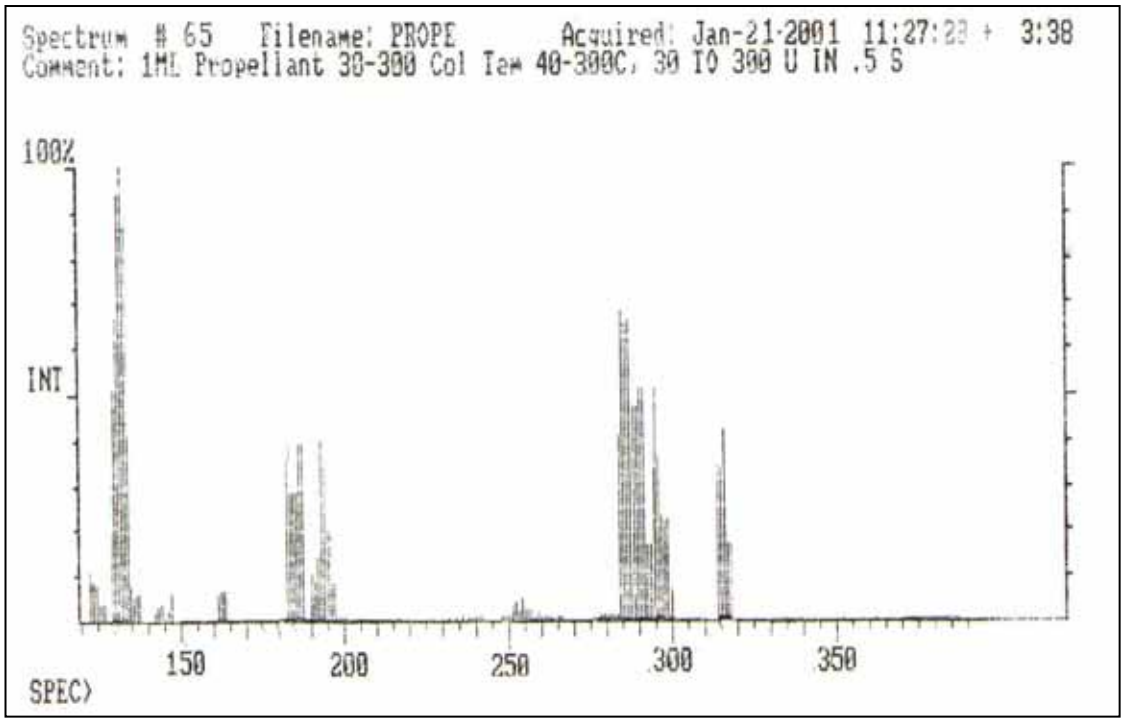


Fig (3-5) Mass spectrum of BIZ azo dye no.17

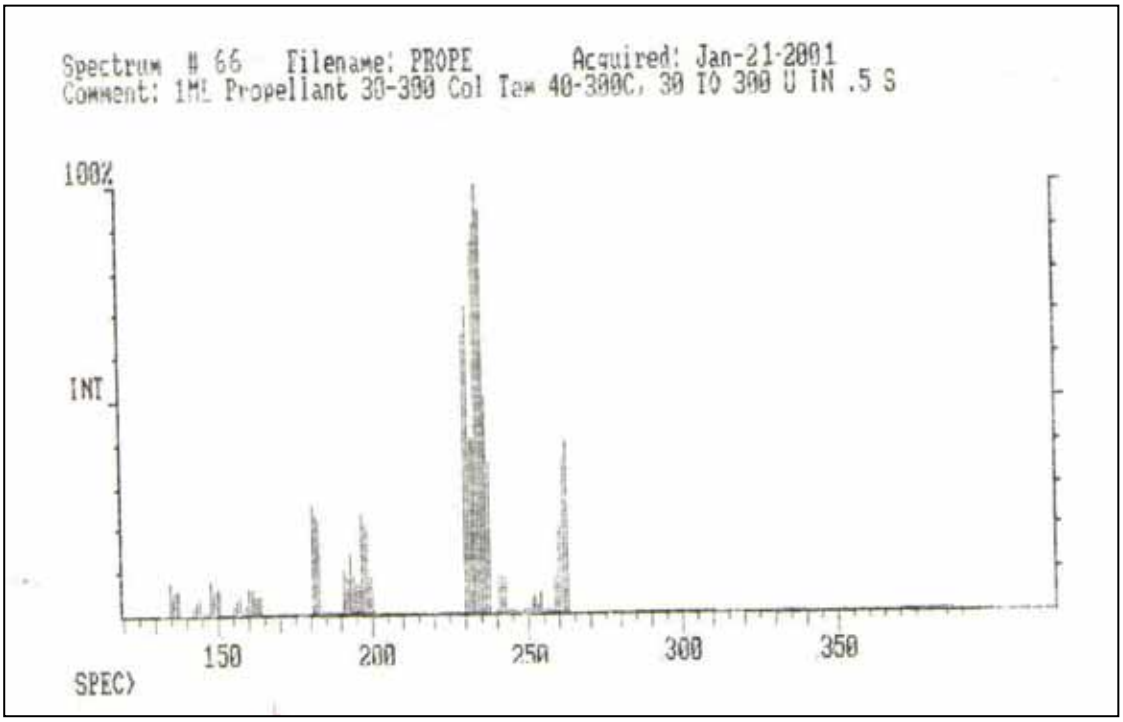
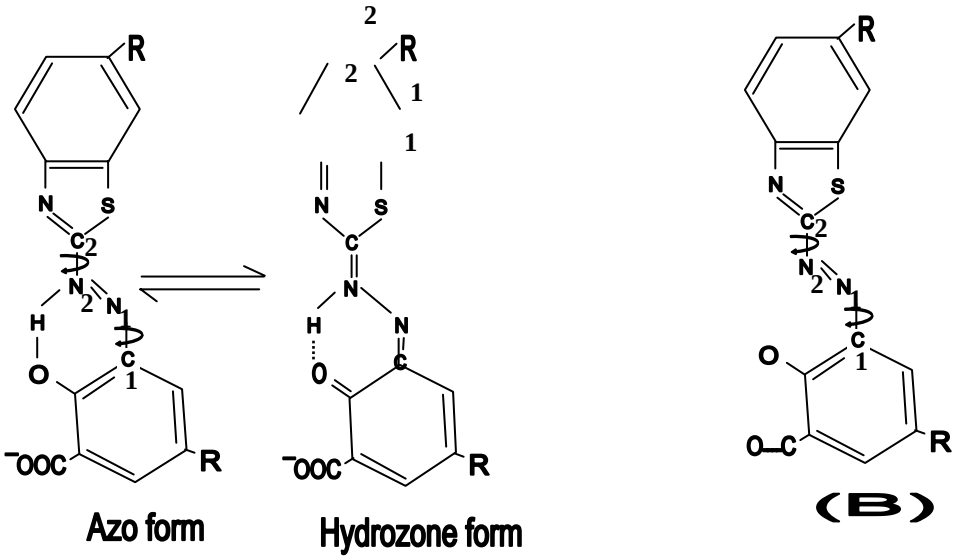


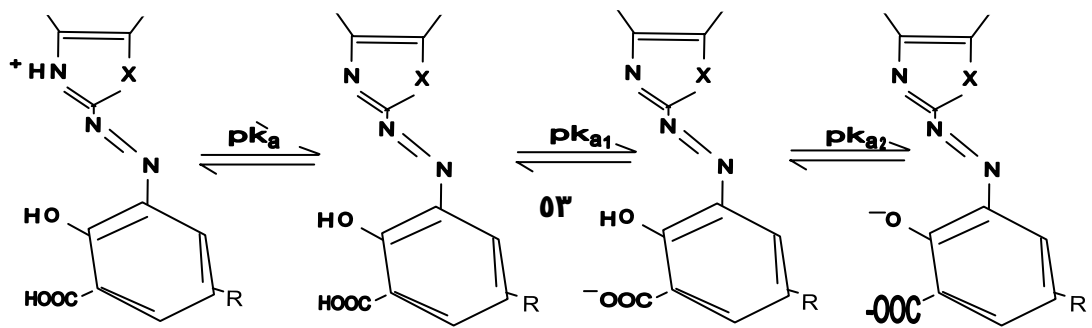
Fig (3-6) Mass spectrum of TZ azo dye no.23

the azo dyes under investigation scheme (3.1A). The hydrogen bond formation leads to marked reduction in the basicity of the nitrogen atom, i.e the formed complex becomes less stable. There is another intramolecular hydrogen bonding that occurs between the carboxylic group and the hydroxyl group in probable position to form six-member ring. In this case, two intramolecular hydrogen- bonding are possibly operational at the same time in the molecule scheme (3,1B). These phenomena leads to a marked



(A) Scheme (3.1) (B)

Decrease in the stability constant. From Uv. and Vis. absorption spectra of the dyes as a function of pH, it was found that dyes show



hypsochromic and batho chromic shifts on protonation and ionization, respectively as in Scheme (3-2).

Protonated form Neutral form Anion form Di anion form

Table (3-1) the fragmentations of some azo dyes mass spectra indicated in fig (3-3 to 3-6)

Azo dye no.	Fragment	M/Z	Relative abundance
BTZ(2)	Parent ion	338	11
	$C_{16}H_{10}N_2O_3S^+$	310	4
	$C_8H_4N_2O_3^+$	176	24
	$C_8H_8N_2S^+$	164	100
	$C_8H_9S^+$	137	12
BTZ(10)	Parent ion	379	26
	$C_{14}H_6N_2O_5S^+$	351	4
	$C_7H_5N_3O_2S^+$	195	18
	$C_7H_3NO_2Cl^+$	185	100
	$C_7H_7S^+$	123	10
BIZ(17)	Parent ion	317	10
	$C_{14}H_9N_2O_3Cl^+$	289	38
	$C_7H_4NO_3Cl^+$	185	21
	$C_7H_6N_3^+$	132	100
TZ(23)	Parent ion	263	30
	$C_{10}H_9N_3O_4^+$	235	100
	$C_8H_7NO_4^+$	181	19

3.3 Stability Constant of the Complex: -

The stability constants of 1:1 complexes of BTZ , BIZ and TZ azo derivatives with UO_2(II), Pb (II), Ce (III), La (III), In(III), Zn (II), Cu (II) and Ni (II) in a media of ions strength (I =0.1 KNO_3)were evaluated . Spectrophotometric method was followed and DALSFASK computational program was used for that purpose [115]. However, no attempt has been made to correlate the stability constants of complexes of BTZ with that of BIZ, both reagents are dibasic acid and simultaneously form the normal type of complexes with most metal ions. BIZ form more stable complexes with metal ions than BTZ because of the lower basicity of the thiazole rings. Ni does not exhibit the same distribution as the other two metals (Cu and Zn) due to the lower preferences for coordination with S than N donar atoms and a much lower ratio for loss of water than Cu and Zn. A comparison was done between TAR – Metal complexes with BTZ – metal complexes and RAR – complexes with BIZ – metal complexes which seem to have the same structure formula and these are listed in Tables (3-3) respectively.

The stability constants of the metal complexes were determined using a computational method, and the values of the stability constants (log K_{ML}) were represented in the Tables (3.2 - 3-4). The following remarks can be achieved:

1.A continuous variation method and mole ratio method were used revealing that (1:1) ML type of complexes were formed in solution.

2. No hydrolysis was observed in solution vessel there fore metal hydroxide formation was excluded.

3.The stability constants of BIZ in metal complexes are higher than the corresponding metal complexes of BTZ, while TZ azo dyes show relatively lower values of (log K_{ML}) than that of BIZ. This is quite reasonable because the BIZ ligands are regarded as better complexing agents, maight due to the basicity of BIZ molecule.

Table (3-3): A comparison between stability constants of similar system

Metal ion	log k_{ML} range			log k_{ML} of TAR [ref .(116-120)]	log k_{ML} of PAR [ref .(121-123)]
	BTZ	BIZ	TZ		
UO_2II	(7.4-8.8)	(8.5-9.2)	(4.3-5.3)	10.9	12.5
Pb II	(7.6-8.0)	(7.8-8.3)	(3.0-3.8)	8.34	11.9
Ce III	(8.5-9.2)	(10.0-11.6)	(6.3-6.7)	7.44	8.0
La III	(6.0-7.7)	(8.5-9.2)	(4.8-5.9)	6.93	8.9
In III	(9.0-9.8)	(11.0-12.7)	(5.3-5.9)	10.1	9.6
Zn II	(6.4-7.9)	(6.5-7.1)	(3.7)	7.30	11.2
Cu II	(7.7-8.7)	(8.8-9.2)	(6.5-7.1)	10.0	17.3
Ni II	(7.6-8.1)	(7.5-8.0)	(5.9-6.3)	9.34	14.1

4.The stability constants of metal complexes of BTZ follow the sequences:

In(III) > Ce(III) >Cu(II) > UO_2(II) > Ni(II) > Pb(II)> Zn (II) > La(III)

For BIZ metal complexes follow the sequences:

In(III) > Ce(III) >Cu(II) > UO2(II) > La(III)> Pb(II) > Ni(II) >Zn(II)

And for TZ metal complexes follow the sequences:

Ce(III) > Cu(II) > Ni(II) > In(III) > La(III) > UO_2(II) > Pb(II) > Zn(II)

The higher values of the stability constants of the M^{3+} complexes are expected on the basis of the charge / ionic radius and ionization potential of the metal ion mostly in the octahedral field environment.

A major factor, which might influence the stability of TZ complexes relative to BIZ and BTZ, is the extent of π- bonding interactions. π-bonding will strengthen the metal ligand linkage. Probably the most important aspect of the back donation to π-bonding is the reduction of the net negative charge donated to the metal. The more electron density the π-system can transfer to the ligand, the more the metal can accept via the σ bond[16]. In turn, the more the σ bond interactive removes electron density from the ligand, the more readily the ligand can accept electron density through the π- system. So such a system can increase the bonding possibilities of the others. This is sometimes called the synergic effect. The reduction in the stability of TZ – metal complexes compared to BIZ and BTZ could be due to the replacement of the benzene rings by imidazole poorer π- acceptor, With the Pb and Zn –TZ derivatives (azo dyes no, 20, 21 with Pb(II) and azo dyes no. 20, 21 and 22 for Zn(II) no complexes were detected pectrophotometrically which maight reveal to the strength of the coordination power because of these ligands, This is in agreement with some expectation because of a reduction in the strength of both σ and π bonding. The ligands under consideration can be placed in the following of decreasing ligand field strength

BIZ > BTZ > TZ

Back donation electron phenomena (M $\longrightarrow$L) π- bonding is expected to occur in these systems and may play a vital part in facilitating

the hydrolysis of water, molecules bond to metal ions. (M $\longrightarrow$L) π-bonding serves to transfer electron density from the metal ion to the ligand. In other words, it tends to increase the net positive charge on the metal ion which will result in an increase in the bond strength between the oxygen of a water molecule and the metal ion, and thus increase in the activity of the water proton.[124]

Many workers [31,115] have introduced a linear relation between the stability constants of metal – ligand complex and the acid ionization constants of the closely related ligands. They are all fundamentally based upon the following equation[30]:

$$\log K_{ML} = pk_{a2} + \frac{1}{2.303RT}((G^{\backslash o}_{HL} - G^{\backslash o}_{ML}) - (G^{\backslash o}_{H} - G^{\backslash o}_{M})) \quad ..(3.1)$$

where G$^{\backslash}$ refers to partial molar free energy.

Providing that the combined molar free energy terms are either negligible, constant or a linear function of pk_{a2}, when series of the corresponding ligands are compared using the same metal, the terms $(G^{\backslash o}_{H} - G^{\backslash o}_{M})$ is constant for closely related compounds and the same metal ion, the terms $(G^{\backslash o}_{HL} - G^{\backslash o}_{ML})$ is unlikely to be zero, so slope of such a plot will be equal to unity or different from unity. The dependence of $(G^{\backslash o}_{HL} - G^{\backslash o}_{ML})$ on basicity of ligand and the charge density on the metal ion, as the increasing in basicity of ligand or the decreasing of (z/r) of the metal ion, the difference in $(G^{\backslash o}_{HL} - G^{\backslash o}_{ML})$ shows more dependence on the hydrogen bond compared with metal – ligand bond. In other words, the deviation from the unit slope can be explained in term of π-bonding:

A) If the slope > 1, this means that the metal ion has π - acceptor properties.

B) If the slope < 1, the metal ion behaves as π - donor properties.

The correlation plots of pK_{a2} vs. log K_{ML} are shown in the Fig (3.7-3-.24).A fairly satisfactory linear relation ,as shown in Fig(3.10,3.11,3.13 ,3.16,3.17, 3.18,3.19,and 3.22) while some show acceptable linear relationship as in Fig(3.7,3.9,3.12,3.14 and3.15) while another not obey equation(3.1) relationship as shown in Fig(3.8 and 3.28), no explanation is found till now for Pb(II) complexes, but the inversely relationship in Fig(3.15)maight concluded to the effective of proton complexes.

The values of slope, intercept, coefficient of determination, R-squared and Residual mean square, were obtained by using GRAPHER and SPSS computer programs with the aid of least squares method to data given in Fig (3.7-3.24) since the slope of the correlation mostly was less than one unity, this indicate that substituents effect the stability of the proton complexes to a greater extent than that of the corresponding metal complexes Fig (3.15-3.26), and that the metal mostly would behave as π donor properties

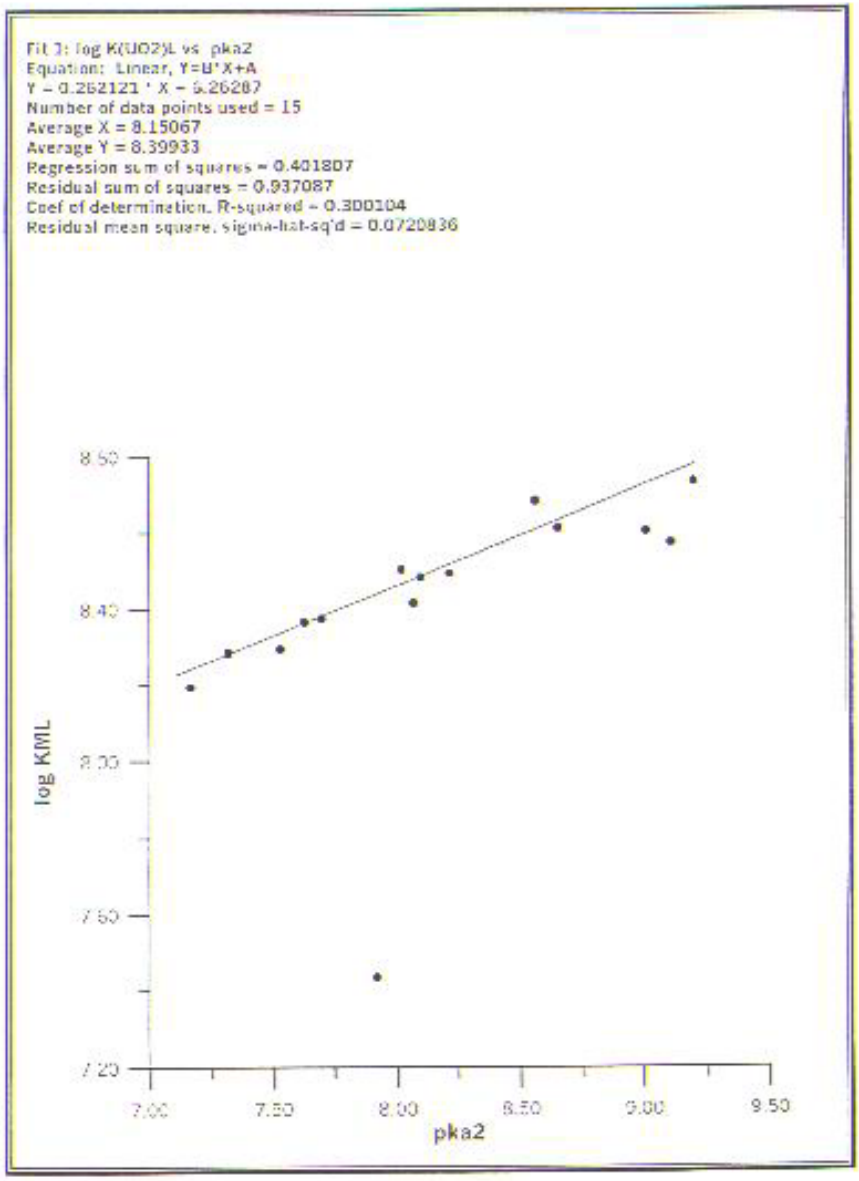


Fig . (3-7):Plot of log K_{ML} (UO_2(II))vs . pka2 of BTZ azo dyes derivatives , for the data in Table(3-2)

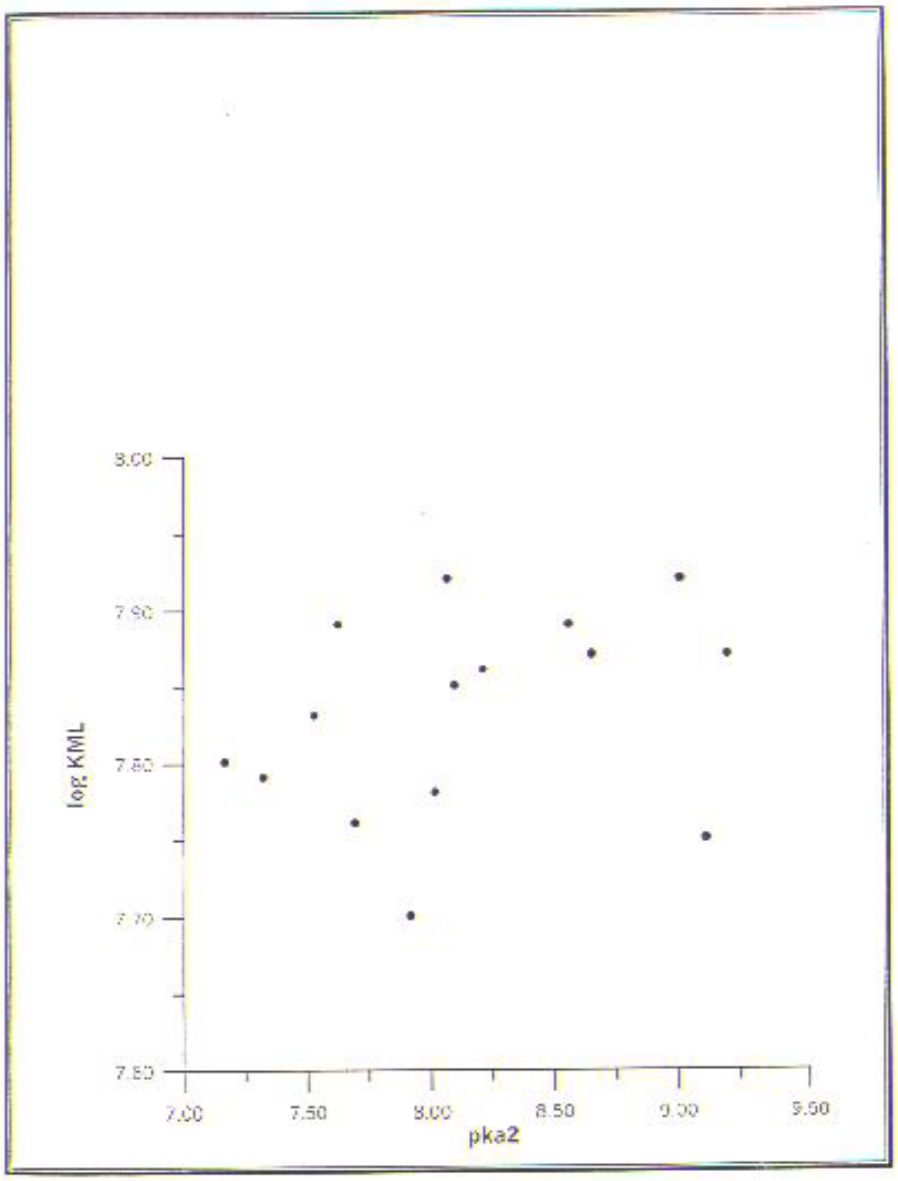


Fig (3-8) **Plot of log K_{ML} (Pb(II)) vs . pka_2 of BTZ azo dyess derivatives for the data in** Table (3-2)

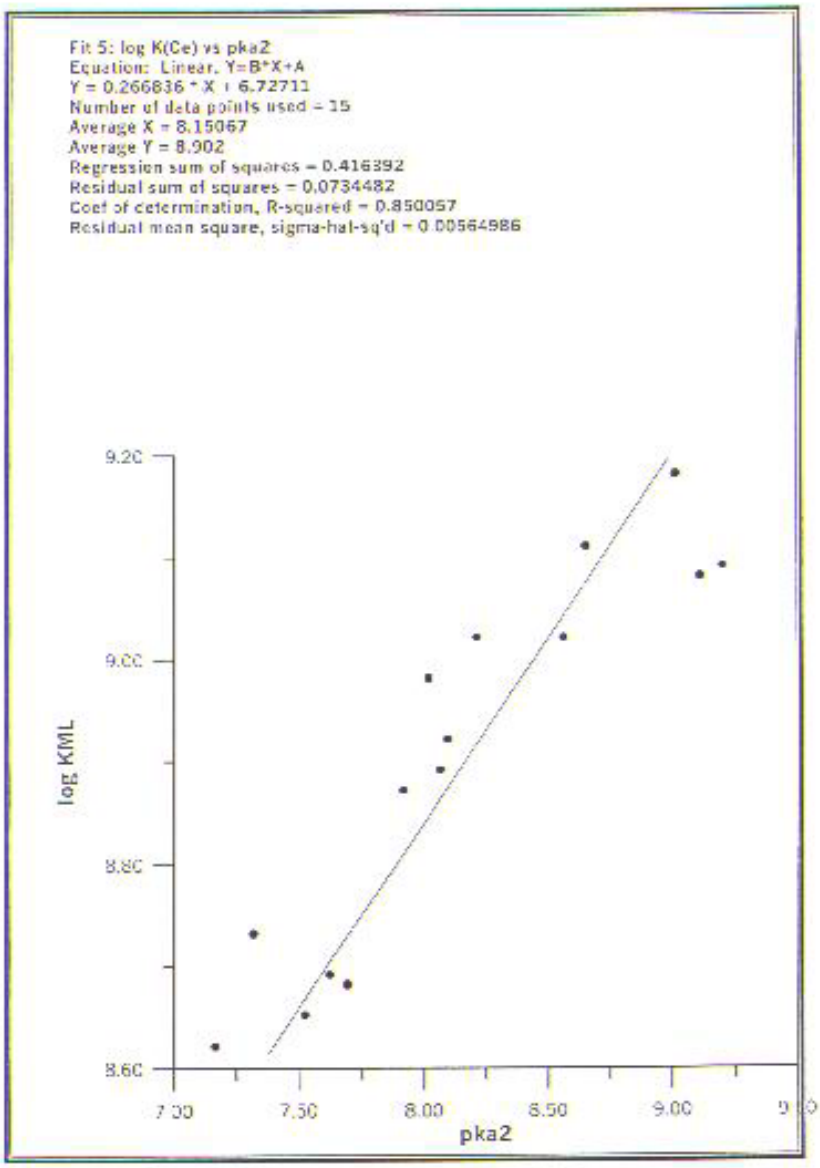


Fig (3-9) :Plot of log K_{ML} (Ce(III)) vs . pka_2 of BTZ azo dyes derivatives for the data in Table (3-2)

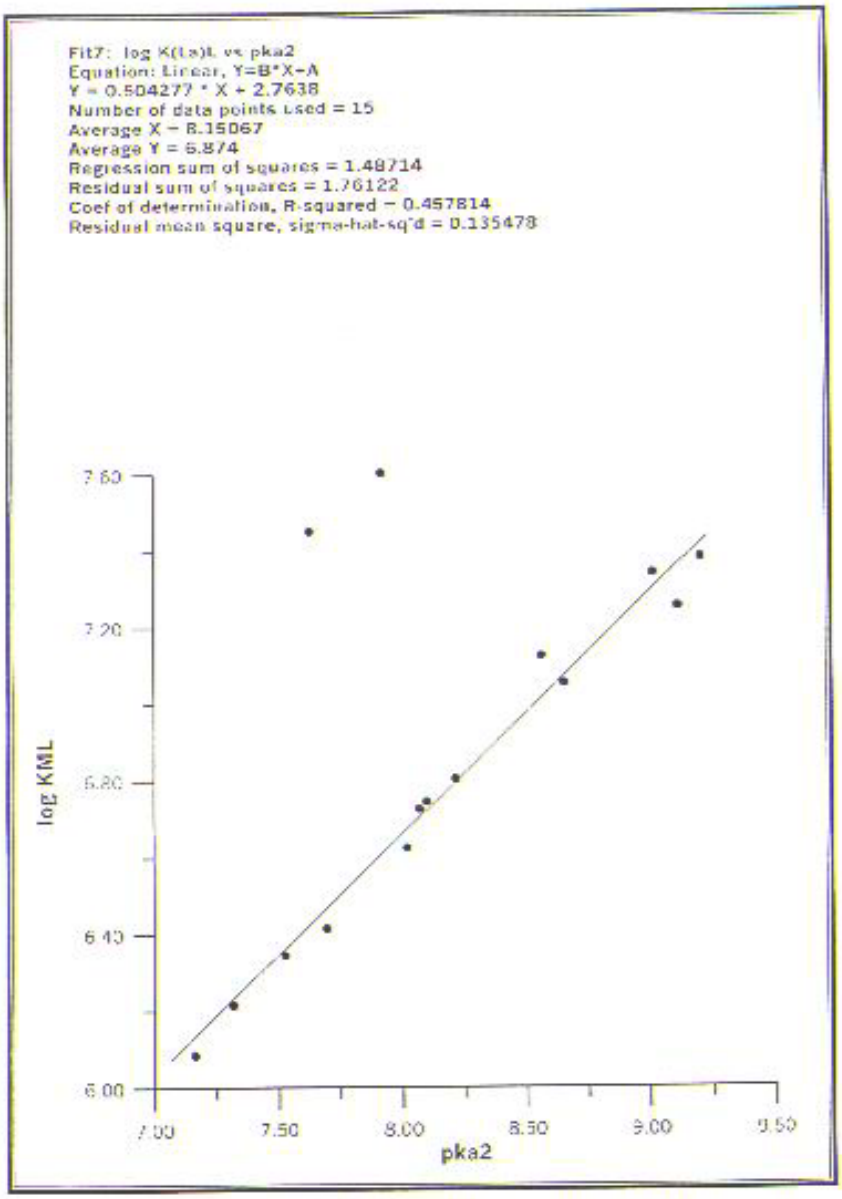


Fig (3-10) :**Plot of log K_{ML} (La(III)) vs . pka2 of BTZ azo dyes derivatives for the data in** Table (3-2)

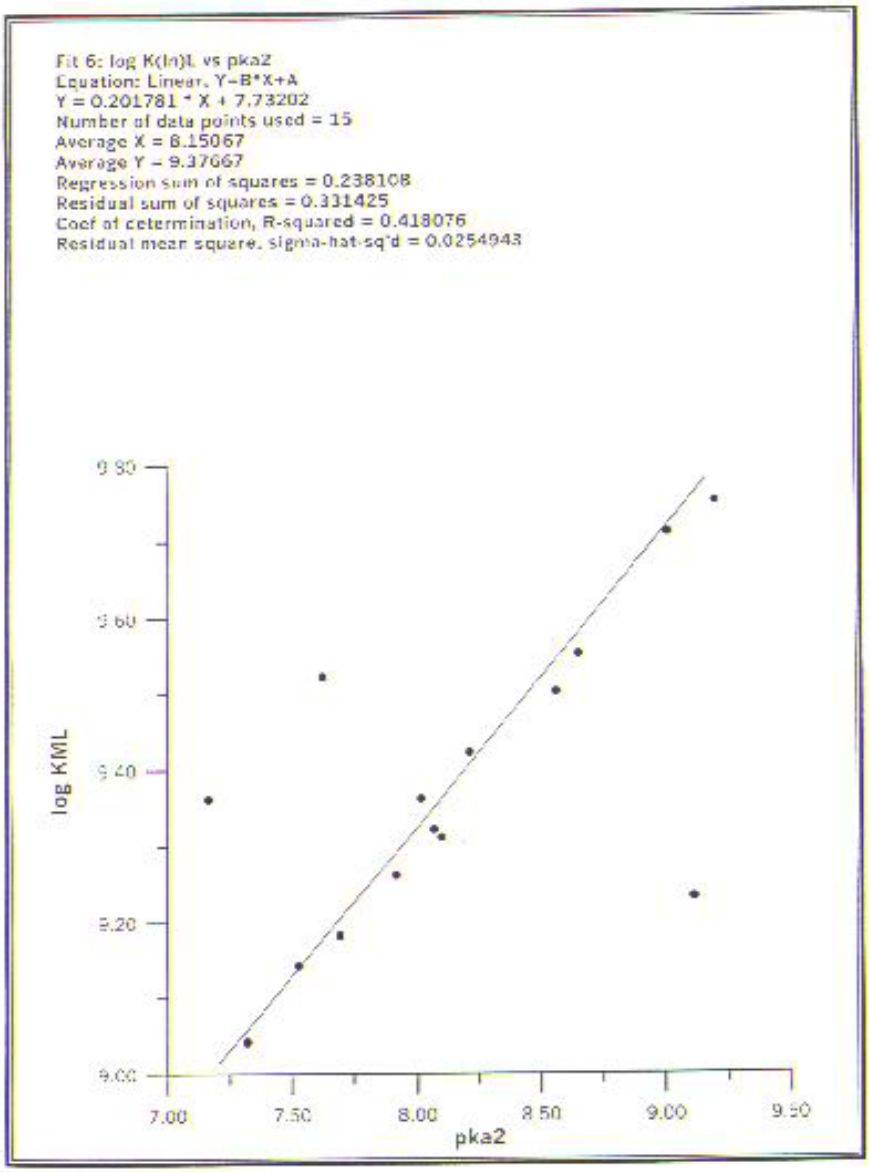


Fig (3-11) sPlot of log K_{ML} (In(III)) vs . pka2 of BTZ azo dyes derivatives for the data in Table (3-2)

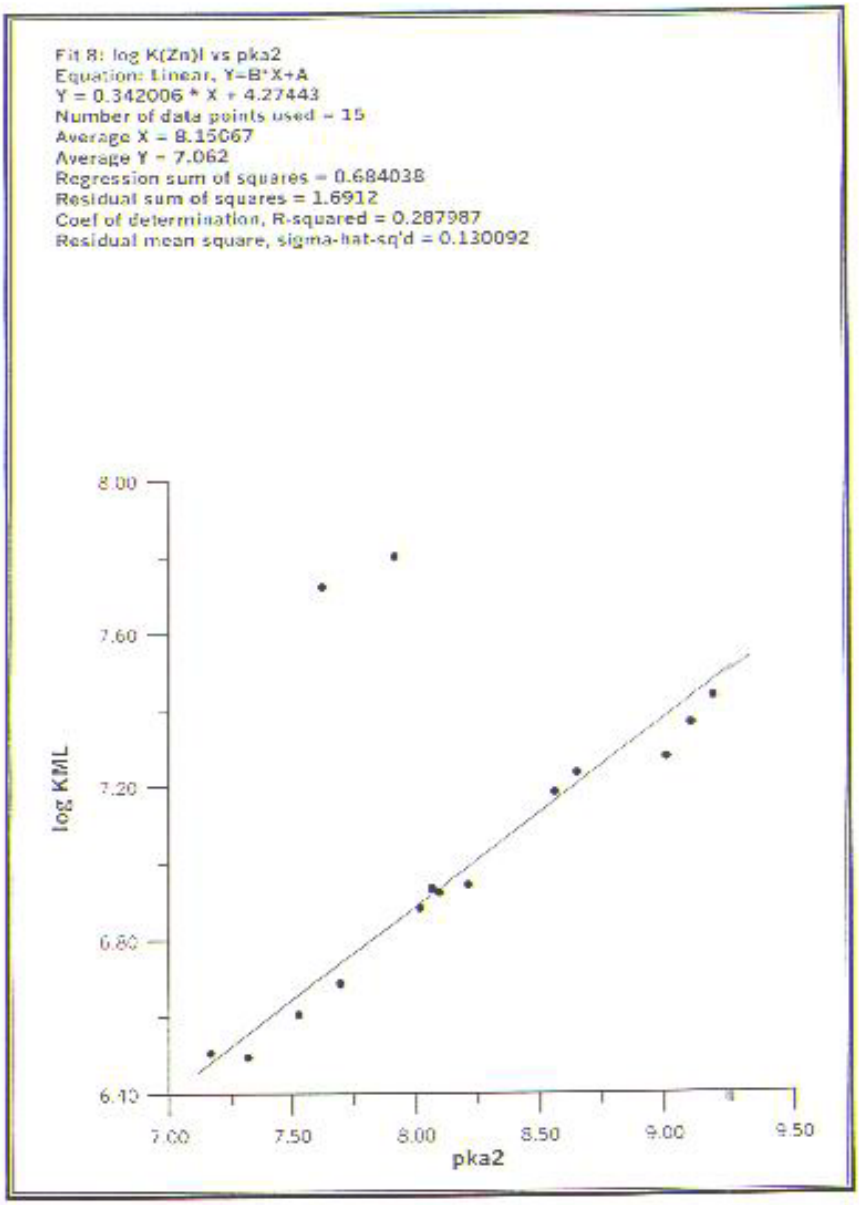


Fig (3-12) :Plot of log K_{ML} (Zn(II)) vs . pka_2 of BTZ azo dyes derivatives for the data in Table (3-2)

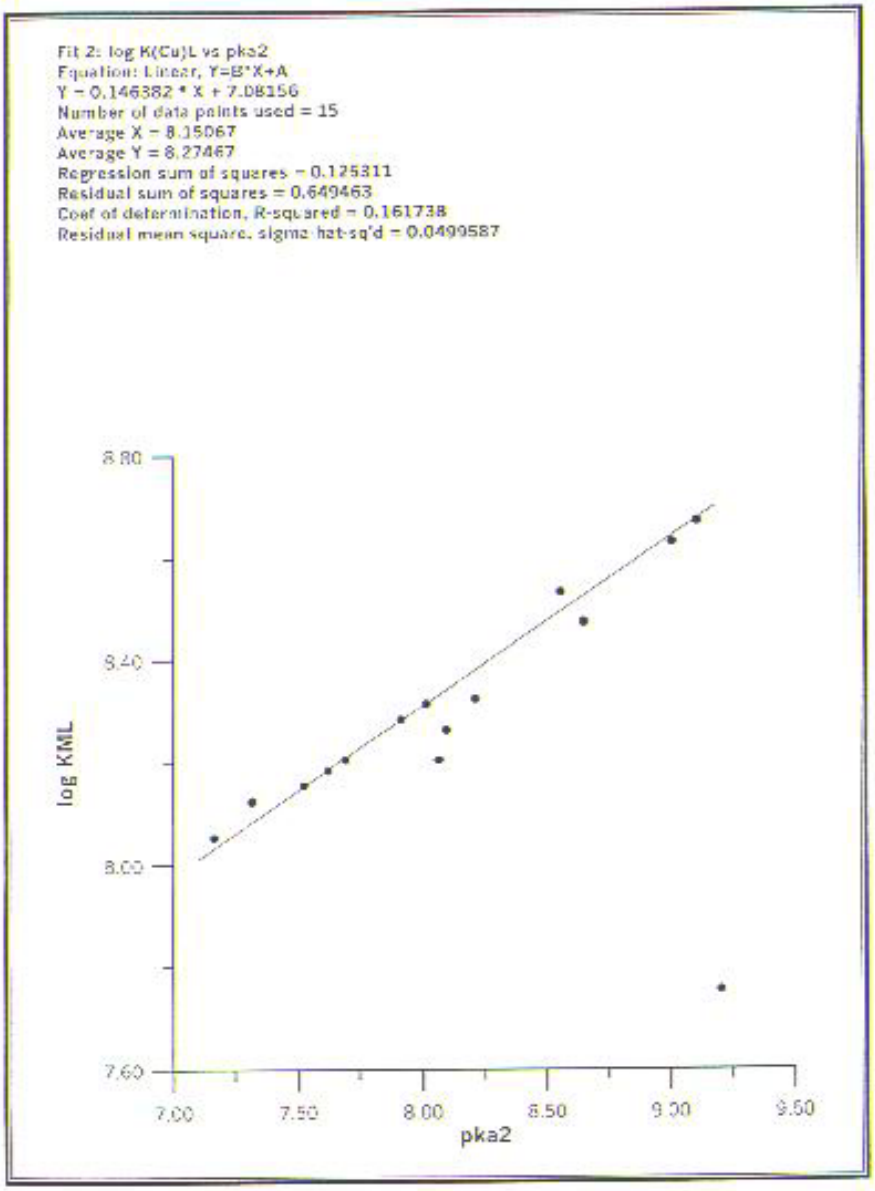


Fig (3-13) :Plot of log K_{ML} (Cu(II)) vs . pka_2 of BTZ azo dyes derivatives for the data in Table (3-2)

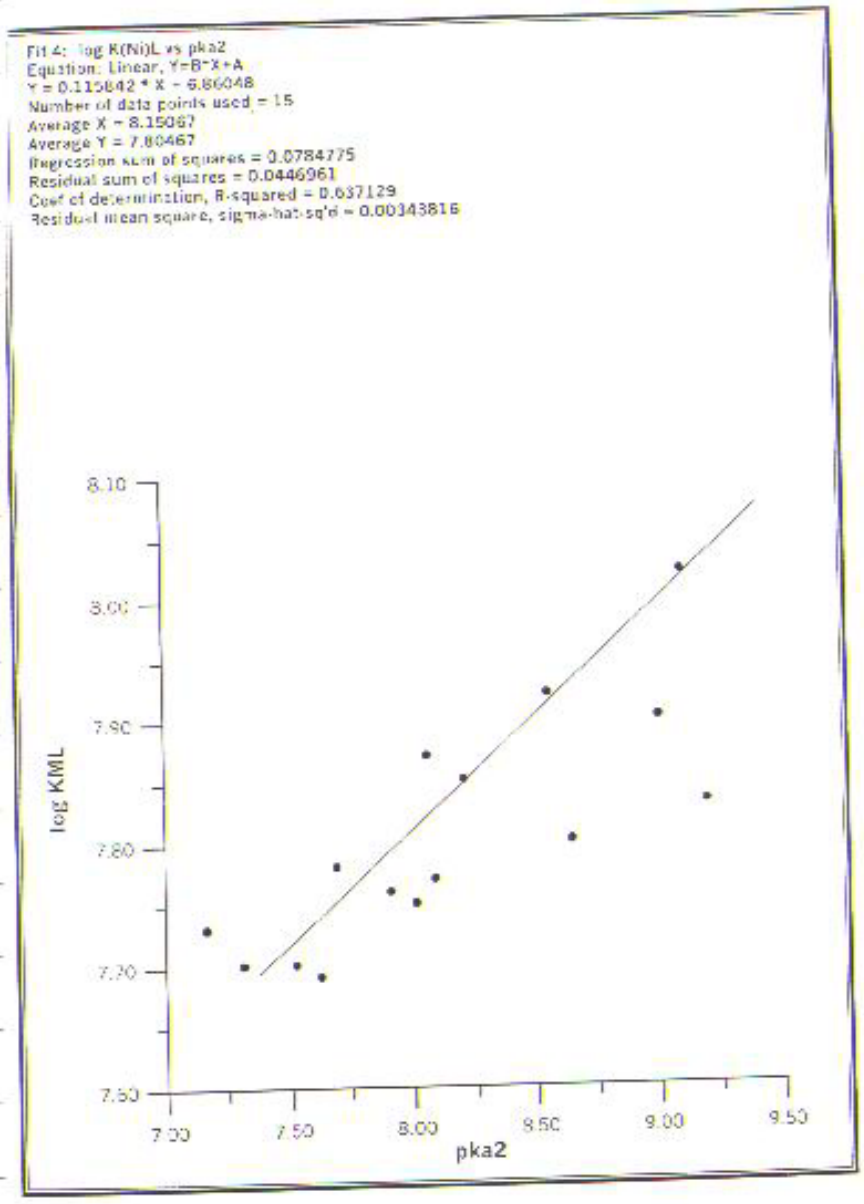


Fig (3-14) :Plot of log K_{ML} (Ni(II)) vs . pka_2 of BTZ azo dyes derivatives for the data in Table (3-2)

Table (3-4) Collective data of thermodynamic stability constants of metal BIZ and TZ azo dyes and their ionization constants

Azo.dye no.	K_{ML}									
	pka2	pka1 +pka2	UO_2(II)	Pb(II)	Ce(III)	La(III)	In(III)	Zn(II)	Cu(II)	Ni(II)
16	8.32	11.95	9.03	8.53	10.68	7.73	8.02	6.78	8.9	11.62
17	10.80	14.70	8.68	9.33	11.52	7.95	8.43	7.05	9.27	12.64
18	10.10	13.84	8.84	9.15	11.3	7.9	8.2	6.76	9.17	12.2
19	7.21	10.04	9.13	8.01	10.07	7.61	7.85	6.55	8.84	11.06
20	7.35	10.25	4.37	0	6.54	5.38	5.98	4.89	0	6.36
21	8.92	12.24	4.5	0	6.69	5.46	6.11	5.37	0	6.48
22	10.00	13.23	4.48	0	6.83	5.68	6.23	5.4	3	6.66
23	11.90	15.41	4.6	3.74	7.03	5.8	6.21	5.85	3.8	6.87

Fit 1: log K(UO2)L vs pka2
Equation: Linear, Y=B*X+A
Y = -0.120281 * X + 10.0155
Number of data points used = 4
Average X = 9.1075
Average Y = 8.92
Regression sum of squares = 0.116757
Residual sum of squares = 0.0034433
Coef of determination, R-squared = 0.971354
Residual mean square, sigma-hat-sq'd = 0.00172165

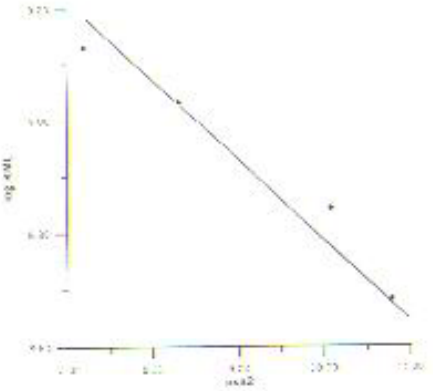
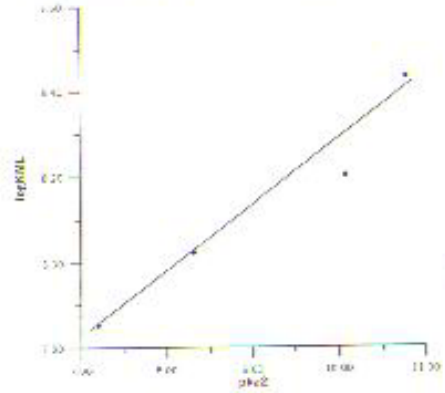


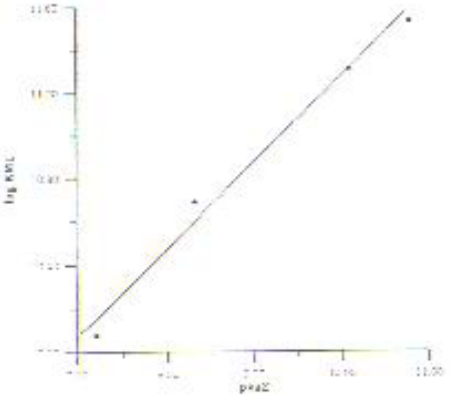
Fig (3- 15) Plot of log K_{ML} (UO_2(II)) vs . pka_2 of BIZ azo dyes derivatives for the data in table **Table (3-4)**

Fit 6: log K(Pb)L vs pka2
Equation: Linear, Y=B*X+A
Y = 0.145208 * X + 6.27624
Number of data points used = 4
Average X = 9.1075
Average Y = 8.125
Regression sum of squares = 0.176993
Residual sum of squares = 0.00620691
Coef of determination, R-squared = 0.966171
Residual mean square, sigma-hat-sq'd = 0.00155346



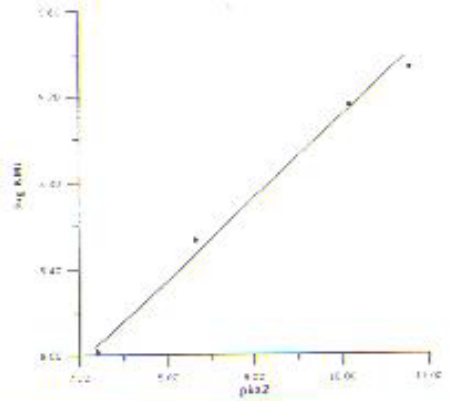
Fig(3- 16) :Plot of log K_{ML} (Pb(II)) vs . pka_2 of BIZ azo dyes derivatives for the data in Table (3-4)

Fit 5: log K(Ce) I vs pka2
Equation: Linear, Y=B*X+A
Y = 0.395839 * X + 7.2674
Number of data points used = 4
Average X = 9.1075
Average Y = 10.8925
Regression sum of squares = 1.76452
Residual sum of squares = 0.0169593
Coef of determination, R-squared = 0.986766
Residual mean square, sigma-hat-sq'd = 0.00847967



Fig(3-17) :Plot of $\log_{10} K_{ML}$ (Ce(III)) vs . pka_2 of BIZ azo dyes derivatives for the data in **Table (3-4)**

Fit 3: log K(La) I vs pka2
Equation: Linear, Y=B*X+A
Y = 0.366289 * X + 5.41001
Number of data points used = 4
Average X = 9.1075
Average Y = 8.755
Regression sum of squares = 1.00237
Residual sum of squares = 0.00993242
Coef of determination, R-squared = 0.991273
Residual mean square, sigma-hat-sq'd = 0.00476621



Fig(3- 18) :Plot of $\log_{10} K_{ML}$ (La(III)) vs . pka_2 of BIZ azo dyes derivatives for the data in **Table (3-4)**

A further means of studying the effect of substitution, correlation between log k_{ML} and (pk_{a1}+pk_{a2}) was derived by Ernst and Menachi [125]

$$\log K_{ML} = \beta(pk_{a1}+pk_{a2}) + \alpha \quad \ldots\ldots..(3.2)$$

Equation (3.2) produces that a plot of log k_{ML} against $pK_{a1} + pK_{a2}$ should yield a straight line for a series of closely related ligands.

Hence, if a linear relationship is hold, three cases are distinguished:

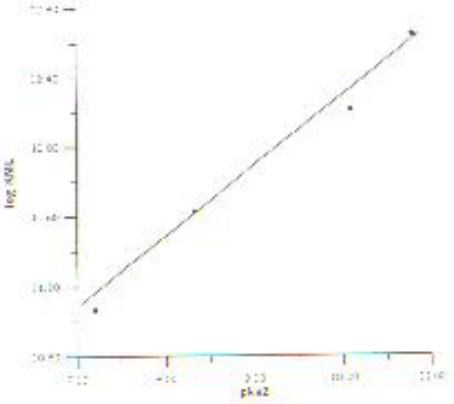
1.slope = 1 ; substitution affects the stability of the metal complexes to the same extent as that of the corresponding proton complexes.

2. slope >1 ; substitution affects the stability of the metal complexes to a greater extent than that of the corresponding proton complexes.

3.slope < 1 ; substitution affects the stability of the metal complexes to a lesser extent than that of the corresponding proton complexes.

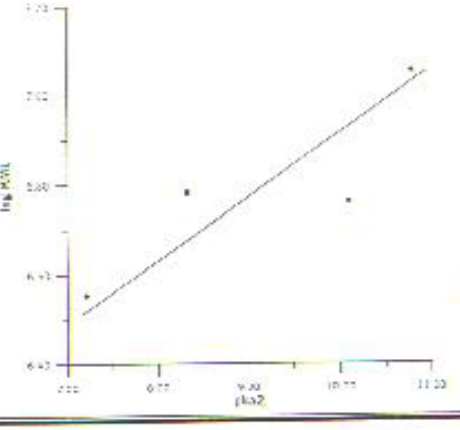
Tables (3.3-3.5) represent the statistical results obtained by plotting of log K_{ML} vs . pk_{a1}+pk_{a2} for the complexes of various metal ions with both BTZ, BIZ and TZ azo dyes. The values of slope, intercept, coefficient of determination, R-squared and Residual mean square, obtained by using GRAPHER and SPSS computer programs with the aid of least squares method to data are given in Tables (3.5-3.7)

Fit 4: log K(In)L vs pka2
Equation: Linear, Y=B*X+A
Y = 0.416913 * X + 8.08297
Number of data points used = 4
Average X = 9.1075
Average Y = 11.88
Regression sum of squares = 1.40274
Residual sum of squares = 0.0172557
Coef of determination, R-squared = 0.987848
Residual mean square, sigma-hat-sq'd = 0.00862783



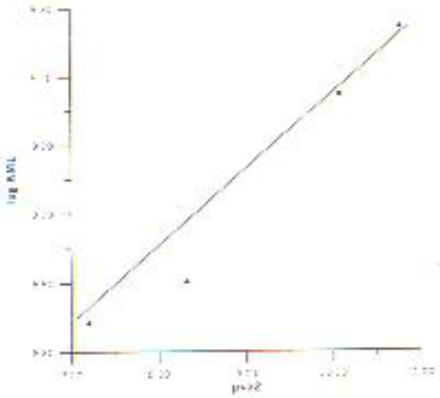
Fig(3- 19) : Plot of log K_{ML} (In(III)) vs . pka_2 of BIZ azo dyes derivatives for the data in **Table (3-4)**

Fit 8 : Log K(Zn)L vs pka2 Equation: Linear, Y=B*X+A
Y = 0.103243 * X + 5.79918
Number of data points used = 4
Average X = 9.1075
Average Y = 6.735
Regression sum of squares = 0.0945556
Residual sum of squares = 0.0315444
Coef of determination, R-squared = 0.749846
Residual mean square, sigma-hat-sq'd = 0.0157722



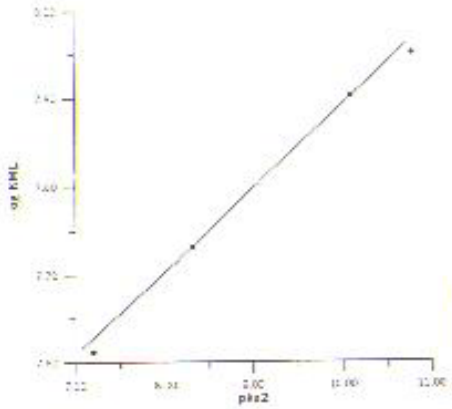
Fig(3- 20) ;Plot of log K_{ML} (Zn(II)) vs . pka_2 of BIZ azo dyes derivatives for the data in **Table (3-4)**

Fit 2: log K(Cu)L vs pka2
Equation: Linear, Y=B*X+A
Y = 0.124908 * X + 7.90739
Number of data points used = 4
Average X = 9.1075
Average Y = 9.045
Regression sum of squares = 0.125915
Residual sum of squares = 0.00338554
Coef of determination, R-squared = 0.973817
Residual mean square, sigma-hat-sq'd = 0.00169277



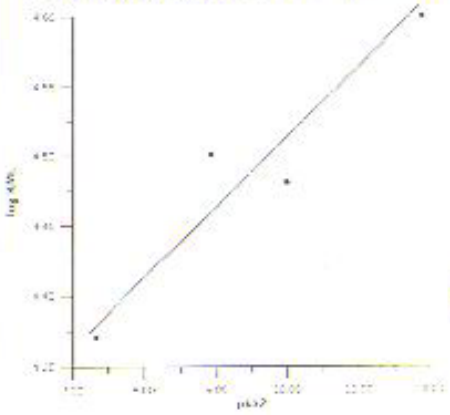
Fig(3- 21) :Plot of log K_{ML} (Cu(II)) vs . pka_2 of BIZ azo dyes derivatives for the data in table **Table (3-4)**

Fit 2: log K(Ni)L vs pka2
Equation: Linear, Y=B*X+A
Y = 0.09526 * X + 6.92592
Number of data points used = 4
Average X = 9.1075
Average Y = 7.7975
Regression sum of squares = 0.0732335
Residual sum of squares = 0.00024144
Coef of determination, R-squared = 0.996714
Residual mean square, sigma-hat-sq'd = 0.00012072



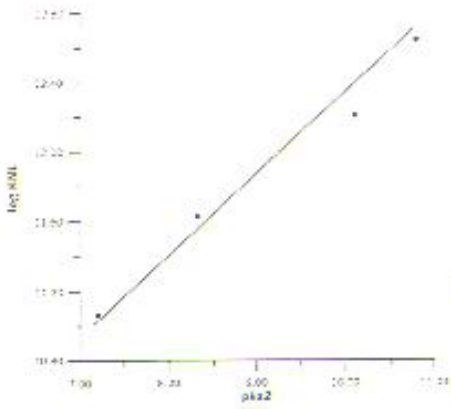
Fig(3- 22) :Plot of log K_{ML} (Ni(II)) vs . pka_2 of BIZ azo dyes derivatives for the data in **Table (3-4)**

Fit 1: log K(UO2)L vs pka2
Equation: Linear, Y=B*X+A
Y = 0.046574 * X + 4.04211
Number of data points used = 4
Average X = 9.5425
Average Y = 4.4875
Regression sum of squares = 0.0238796
Residual sum of squares = 0.00279543
Coef of determination, R-squared = 0.895204
Residual mean square, sigma-hat-sq'd = 0.00139771



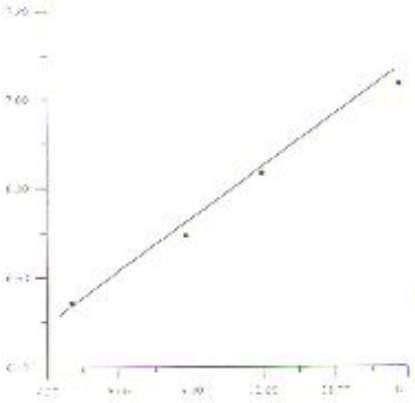
Fig(3- 23) :Plot of log K_{ML} (UO_2(II)) vs . pka_2 of TZ azo dyes derivatives for the data in **Table (3-4)**

Fit 2: log K(Ce)L vs pka2
Equation: Linear, Y=B*X+A
Y = 0.416913 * X + 8.08297
Number of data points used = 4
Average X = 9.1075
Average Y = 11.88
Regression sum of squares = 1.40274
Residual sum of squares = 0.0172557
Coef of determination, R-squared = 0.987848
Residual mean square, sigma-hat-sq'd = 0.00862783



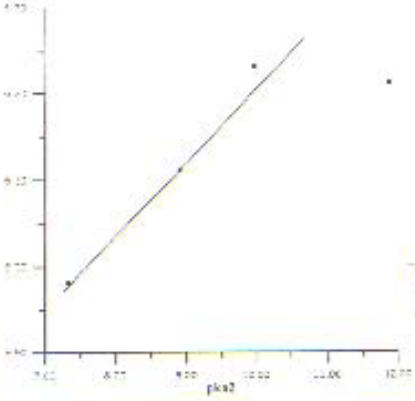
Fig(3- 24) :Plot of log K_{ML} (CeIII) vs . pka_2 of TZ azo dyes derivatives for the data in **Table (3-4)**

Fit 5: log K(Cu)l vs pka2
Equation: Linear, Y=B*X+A
Y = 0.108968 * X + 5.73267
Number of data points used = 4
Average X = 9.5425
Average Y = 6.7725
Regression sum of squares = 0.13016
Residual sum of squares = 0.000315097
Coef of determination, R-squared = 0.997585
Residual mean square, sigma-hat-sq'd = 0.000157549



Fig(3- 25) :Plot of log K_{ML} ((Cu(II)) vs . pka2 of TZ azo dyes derivatives for the data in **Table (3-4)**.

Fit 4: log K(Ni)l vs pka2
Equation: Linear, Y=B*X+A
Y = 0.0525171 * X + 5.63136
Number of data points used = 4
Average X = 9.5425
Average Y = 6.1325
Regression sum of squares = 0.0302328
Residual sum of squares = 0.00904224
Coef of determination, R-squared = 0.769771
Residual mean square, sigma-hat-sq'd = 0.00452112



Fig(3- 26) :Plot of log K_{ML} ((Ni(II)) vs . pka2 of TZ azo dyes derivatives for the data in **Table (3-4)**

3.4 Structural Study of the Complexes: -

A series of azo dyes derivatives were studied previously by IR and H-NMR spectroscopy. The compounds studied and dissolved [41] in $CDCl_3$ solution proved the presence of intermolecular hydrogen bonds as preferred flattened chair- chair conformation or Zig-Zag form [42].

A structural study[(126)] of copper –2 amino Benzothiazole complex in presence of formate ions showed that Cis bis(2- amino bezothizaole)bis formate copper[11] was formed, by using X-ray technique, it was confirmed that the complex has a distorted octahedral with Six coordination number.

The four closest donor atoms were two N atoms of 2-amino BTZ and two oxygen atoms of the carboxylate groups. They form a distorted square-planner arrangement with Cu-N and Cu-O distances of 1.991A^0 and 1.993 A^o respectively,

Maura *et.al* [127] concluded that the hydrogen atoms of each amino group of 2-amino benzothiazole molecules forms inter – and intra-molecules hydrogen bonds with oxygen atoms from the acetate groups. Inter-molecular S-S secondary bonding of 3.502 A^0 occurred between neighboring chains of molecules connected by hydrogen bonds.

Zhang *et al*. [43] studied the complex configuration Lanthanide of BTZ-2-thiolate with the same ions (Ln(I), Dy(II), Sm(I), and Y (III). All these complexes have been characterized by elemental analysis, IR, X-ray crystallography, and mass spectra. The crystal structural analysis shows that these ions coordinate with BTZ-2-thiolate to form a distorted trigonal bipyramidal geometry with coordination number five. The Ln-S bond length in the longest values was found in organo lanthanide complexes.

For a multidentate ligands such as azo dyes in this investigation; there are two main factors that influence the structure of their complexes:

A) Intramolecular hydrogen bonding as discussed in section (3-2).

B) The variation in the attachment of azo group to the benzene rings with its hard oxygen atom and relatively hard nitrogen donor atom will be able to form relative strong complexes with metal ion, which lie in the bordar line region between hard and soft acceptors. Azo dyes derivatives seem to react with metal and behaveas tridentate ligands enolate attached to the azo nitrogen adjusted to the phenol ring, oxygen of phenol group and thiazole nitrogen atom therefore, two five members chelate ring, no example participation of the thiazole sulfur atom in metal bonding was found [34]. Reagents of this class behave as tridentate ligands, giving colored (red or red-violet) chelate with metal ions under study, in acidic and slightly acidic solutions form ML type chelate. In alkaline solution, the hydrolysis will occur; therefore at this rang of pH, hydrolysis of the metal ions must be avoided. In case of BIZ and TZ azo dyes, these type of ligands were assumed[10] to bind through thier pyridyl nitrogen and not through the imidazole nitrogen. According to the azo – hydrazone tautomerism of BTZ compounds [128-129], both BTZ

Fit 1: Linear, Y=B*X+A
Equation:
Y = 0.170647 * X + 6.55612
Number of data points used = 15
Average X = 10.8013
Average Y = 8.39933
Regression sum of squares = 0.285461
Residual sum of squares = 1.05343
Coef of determination, R-squared = 0.213206
Residual mean square, sigma-hat-sq'd = 0.0810333B

a: Plot of log K_{SH} (UO_2) vs. $pk_{a1} + pk_{a2}$

Fit 3: Linear, Y=B*X+A
Equation:
Y = 0.0214184 * X + 7.60065
Number of data points used = 15
Average X = 10.8013
Average Y = 7.832
Regression sum of squares = 0.00449699
Residual sum of squares = 0.056543
Coef of determination, R-squared = 0.0736729
Residual mean square, sigma-hat-sq'd = 0.00434946D

b: Plot of log K_{SH} (Pb) vs. $pk_{a1} + pk_{a2}$

Fit 5: Linear, Y=B*X+A
Equation:
Y = 0.175079 * X - 7.01091
Number of data points used = 15
Average X = 10.8013
Average Y = 8.902
Regression sum of squares = 0.300482
Residual sum of squares = 0.189359
Coef of determination, R-squared = 0.613427
Residual mean square, sigma-hat-sq'd = 0.0145661F

c: Plot of log K_{SH} (Ce) vs. $pk_{a1} + pk_{a2}$

Fit 7: Linear, Y=B*X+A
Equation
Y = 0.368214 * X + 2.8968
Number of data points used = 15
Average X = 10.8013
Average Y = 6.874
Regression sum of squares = 1.32908
Residual sum of squares = 1.91928
Coef of determination, R-squared = 0.409153
Residual mean square, sigma-hat-sq'd = 0.147637H

d: Plot of log K_{SH} (La) vs. $pk_{a1} + pk_{a2}$

Fit 6: Linear, Y=B*X+A
Equation
Y = 0.136264 * X + 7.90483
Number of data points used = 15
Average X = 10.8013
Average Y = 9.37667
Regression sum of squares = 0.182017
Residual sum of squares = 0.387516
Coef of determination, R-squared = 0.31959
Residual mean square, sigma-hat-sq'd = 0.0298089g

e: Plot of log K_{SH} (In) vs. $pk_{a1} + pk_{a2}$

Fit 8: Linear, Y=B*X+A
Equation:
Y = 0.252506 * X + 4.3346
Number of data points used = 15
Average X = 10.8013
Average Y = 7.062
Regression sum of squares = 0.625018
Residual sum of squares = 1.75022
Coef of determination, R-squared = 0.263139
Residual mean square, sigma-hat-sq'd = 0.134632I

f: Plot of log K_{SH} (Zn) vs. $pk_{a1} + pk_{a2}$

Fit 2: Linear, Y=B*X+A
Equation:
Y = 0.125608 * X - 6.91793
Number of data points used = 15
Average X = 10.8013
Average Y = 8.27467
Regression sum of squares = 0.154662
Residual sum of squares = 0.620112
Coef of determination, R-squared = 0.199622
Residual mean square, sigma-hat-sq'd = 0.0477009C

g: Plot of log K_{SH} (Cu) vs. $pk_{a1} + pk_{a2}$

Fit 4: Linear, Y=B*X+A
Equation:
Y = 0.0849767 * X + 6.8868
Number of data points used = 15
Average X = 10.8013
Average Y = 7.80467
Regression sum of squares = 0.0707863
Residual sum of squares = 0.0523873
Coef of determination, R-squared = 0.574687
Residual mean square, sigma-hat-sq'd = 0.00402979E

h: Plot of log K_{SH} (Ni) vs. $pk_{a1} + pk_{a2}$

Table (3-5): The statistical results obtained by plotting of log K_{SH} vs . $pk_{a1} + pk_{a2}$ of BTZ [from Table (3-2)

Fit 1: Linear, Y=B*X+A
Equation
Y = -0.000107686 * X + 8.9479
Number of data points used = 4
Average X = 259.082
Average Y = 8.92
Regression sum of squares = 0.00871851
Residual sum of squares = 0.111481
Coef of determination, R-squared = 0.0725334
Residual mean square, sigma-hat-sq'd = 0.0557407

a: Plot of log K_{ML} (UO_2) vs. $pk_{a1} + pk_{a2}$

Fit 6: Linear, Y=B*X+A
Equation
Y = 0.000101334 * X + 8.09875
Number of data points used = 4
Average X = 259.082
Average Y = 8.125
Regression sum of squares = 0.00772038
Residual sum of squares = 0.17758
Coef of determination, R-squared = 0.0416642
Residual mean square, sigma-hat-sq'd = 0.0887899F

b: Plot of log K_{ML} (Pb) vs. $pk_{a1} + pk_{a2}$

Fit 5: Linear, Y=B*X+A
:Equation
Y = 0.000546196 * X - 10.751
Number of data points used = 4
Average X = 259.082
Average Y = 10.8925
Regression sum of squares = 0.224298
Residual sum of squares = 1.05718
Coef of determination, R-squared = 0.175031
Residual mean square, sigma-hat-sq'd = 0.528589E

c: Plot of log K_{ML} (Ce) vs. $pk_{a1} + pk_{a2}$

Fit 3: Linear, Y=B*X+A
:Equation
Y = 0.000529255 * X + 8.61788
Number of data points used = 4
Average X = 259.082
Average Y = 8.755
Regression sum of squares = 0.210599
Residual sum of squares = 0.8817
Coef of determination, R-squared = 0.192803
Residual mean square, sigma-hat-sq'd = 0.44085C

d: Plot of log K_{ML} (La) vs. $pk_{a1} + pk_{a2}$

Fit 4: Linear, Y=B*X+A
:Equation
Y = 0.000430053 * X + 11.7686
Number of data points used = 4
Average X = 259.082
Average Y = 11.88
Regression sum of squares = 0.13905
Residual sum of squares = 1.28095
Coef of determination, R-squared = 0.0979226
Residual mean square, sigma-hat-sq'd = 0.640475D

e: Plot of log K_{ML} (In) vs. $pk_{a1} + pk_{a2}$

Fit 8: Linear, Y=B*X+A
:Equation
Y = -3.20864E-005 * X + 6.79331
Number of data points used = 4
Average X = 259.082
Average Y = 6.785
Regression sum of squares = 0.00077405
Residual sum of squares = 0.125326
Coef of determination, R-squared = 0.00613838
Residual mean square, sigma-hat-sq'd = 0.062663H

f: Plot of log K_{ML} (Zn) vs. $pk_{a1} + pk_{a2}$

Fit 2: Linear, Y=B*X+A
:Equation
Y = 0.000167582 * X - 9.00158
Number of data points used = 4
Average X = 259.082
Average Y = 9.045
Regression sum of squares = 0.0211146
Residual sum of squares = 0.108186
Coef of determination, R-squared = 0.163299
Residual mean square, sigma-hat-sq'd = 0.0540928B

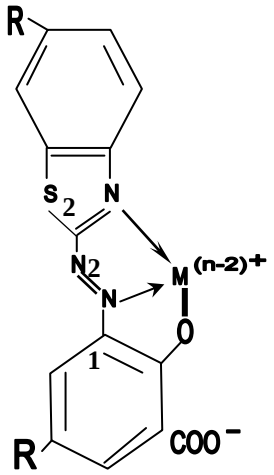
g: Plot of log K_{ML} (Cu) vs. $pk_{a1} + pk_{a2}$

Fit 7: Linear, Y=B*X+A
:Equation
Y = 0.000137341 * X + 7.76192
Number of data points used = 4
Average X = 259.082
Average Y = 7.7975
Regression sum of squares = 0.0141816
Residual sum of squares = 0.0592933
Coef of determination, R-squared = 0.193013
Residual mean square, sigma-hat-sq'd = 0.0296467G

h: Plot of log K_{ML} (Ni) vs. $pk_{a1} + pk_{a2}$

Table (3.6) : The statistical results obtained by plotting of log K_{ML} vs . $pk_{a1}+pk_{a2}$ of BIZ I from **Table (3-2)**

and phenol rings must rotate by 180^0 around the N(2)-C(2) and N(1) – C(1) bonds Scheme(3-1) during the coordination with metal ions. It should be noted that this rotation did not occur if C(1)-N(1) was double bound (i.e. hydrozone form) , therefore, the reaction will occur through the azo form only . as illustrated in scheme (3-3):



Scheme (3-3)

There are several types of metal bonds like M-O, M-azo group and H-M bonds, ΔH^{O} values vary with the nature of the donor and acceptor groups, the medium of the reaction and the presence of neighboring group (i.e steric hindrance), BTZ, BIZ and TZ have identical coordination sites and very similar reaction stereo chemistry with a series of metal ions UO_2(II), Pb (II), Ce (III), La (III), In(III), Zn (II), Cu (II) and Ni (II).

3.5 Thermodynamic Study of the Complex: -

The stability constants of UO_2(II), Pb (II), Ce (III), La (III), In(III), Zn (II), Cu (II) and Ni (II) complexes have been evaluated at 291,298 and 306K^o. The enthalpy change (ΔH^o) for the complexation process was calculated from the slope of the plot (log K_{ML} vs 1/T) by using the least squares method. From the (ΔG^o) and (ΔH^o) values, one can obtain the enthalpy change using the well – known relationships [130] :

$$\Delta G^o = -2.303RT \log K_{ML} \quad \text{.....(3.3)}$$

$$\Delta S^o = (\Delta H^0 - \Delta G^o) / T \quad \text{.....(3.4)}$$

The thermodynamic parameters of the complexation process of BTZ, BIZ and TZ with a various metal ions are summarized in the Tables (3.8-3.15), their result reveal the following:

i) The metal ions exist in solution in a hydrated octahedral form.

ii)The ΔH^o and ΔS^o represent the sum of (1) release of ^+H_2O molecules, and (2) metal-ligand formation.

The metal ions in aqueous solution, where the orientation of water molecules around them during complex formation process between the oppositely charged ions (ligand $\bar{L}$ and M^{x+}) leads to the breakdown of metal – water arrangement, resulting in a positive entropy.

Fit 1: Linear, Y=B*X+A
:Equation
Y = 0.046674 * X + 4.04211
Number of data points used = 4
Average X = 9.5425
Average Y = 4.4875
Regression sum of squares = 0.0238796
Residual sum of squares = 0.00279543
Coef of determination, R-squared = 0.895204
Residual mean square, sigma-hat-sq'd = 0.00139771B

a: Plot of log K_{ML} (UO_2) vs. $pk_{a1} + pk_{a2}$

Fit 4: Linear, Y=B*X+A
:Equation
Y = 0.115391 * X + 5.49139
Number of data points used = 4
Average X = 9.5425
Average Y = 6.5925
Regression sum of squares = 0.145955
Residual sum of squares = 0.00232023
Coef of determination, R-squared = 0.984352
Residual mean square, sigma-hat-sq'd = 0.00116011E

b: Plot of log K_{ML} (Ce) vs. $pk_{a1} + pk_{a2}$

Fit 8: Linear, Y=B*X+A
:Equation
Y = 0.200492 * X + 3.46431
Number of data points used = 4
Average X = 9.5425
Average Y = 5.3775
Regression sum of squares = 0.440626
Residual sum of squares = 0.0208493
Coef of determination, R-squared = 0.95482
Residual mean square, sigma-hat-sq'd = 0.01042471

c: Plot of log K_{ML} (La) vs. $pk_{a1} + pk_{a2}$

Fit 7: Linear, Y=B*X+A
:Equation
Y = 0.0983062 * X + 4.64191
Number of data points used = 4
Average X = 9.5425
Average Y = 5.58
Regression sum of squares = 0.105935
Residual sum of squares = 0.00686528
Coef of determination, R-squared = 0.939138
Residual mean square, sigma-hat-sq'd = 0.0034326411

d: Plot of log K_{ML} (In) vs. $pk_{a1} + pk_{a2}$

Fit 5: Linear, Y=B*X+A
:Equation
Y = 0.108968 * X + 5.73267
Number of data points used = 4
Average X = 9.5425
Average Y = 6.7725
Regression sum of squares = 0.13016
Residual sum of squares = 0.000315097
Coef of determination, R-squared = 0.997585
Residual mean square, sigma-hat-sq'd = 0.0001575491F

e: Plot of log K_{ML} (Cu) vs. $pk_{a1} + pk_{a2}$

Fit 6: Linear, Y=B*X+A
:Equation
Y = 0.0525171 * X + 5.63126
Number of data points used = 4
Average X = 9.5425
Average Y = 6.1325
Regression sum of squares = 0.0302328
Residual sum of squares = 0.00904224
Coef of determination, R-squared = 0.769771
Residual mean square, sigma-hat-sq'd = 0.004521126

f: Plot of log K_{ML} (Ni) vs. $pk_{a1} + pk_{a2}$

Table (3-7): The statistical results obtained by plotting of log K_{ML} vs . $pk_{a1}+pk_{a2}$ of TZ 1from **Table (3-2)**

i) The stability constants log K_{ML} decreased with the increase of temperature, except the log K_{ML} of few complexation process as indicated in Tables (3.8-3.15), which showed an increase of log K_{ML} with the increase of the temperature.

ii) The negative values of ΔG^{o} for complexation process with BTZ, BIZ and TZ suggests a spontaneous nature of such process.

iii) The ΔH^{o} values are negative revealing an exothermic process that is favorable at lower temperatures.

iv) The positive values of ΔS^{o} for the complexation process of BTZ, BIZ and TZ with UO_2(II), Pb (II), Ce (III), La (III), In(III), Zn(II), Cu(II) and Ni(II) confirming that the complex formation process is entropically favorable.[80,81]

Table (3.8-3.15) summarizes the obtained thermodynamic functions for the different members of these series. The lower stability constants of BTZ and various metal ions compared with BIZ complexes and high values of ΔS^{o} provide an evidence to the fact that the steric factor is the more predominating on the BTZ type of complexes. The obtained high values of ΔS^{o} mean most probably that the azo dyes exert to oriented during the complex formation, retorting the attack of metal ion to the complexation site. It is intersting to note that ΔS^{o} of all the members of the BTZ series show high values compared with those of BIZ and TZ complexes, i.e the entropy factor is of a considerable value.

The slightly exothermic values of ΔH^O may be a consequence of formation of a weak metal ion – ligand bond. without displacement of water molecules from the first hydration shell of the cation. It is possible to make a more detailed discussion of ΔH^O values. the latter reflect the changes in the numbers and strengths of the bond made and broken during the ΔH^O to the type of bonding between the metal ion and ligand molecule and to the structural features of the complexes.

So it is convenient to divide (ΔH^O) into two parts, ΔH_e representing long range electrostatic forces depending upon environmental parameter environmental and increasing with the rise of temperature, and ΔH_c representing short – range covalent forces insensitive to environmental and independent of temperature changes. The data in table (3.8-3.15) illustrate the ΔH_c and ΔH_e for the metal – ligand under investigation.

The effect of coordination to a metallic cation on the acid strength of a group on a ligand bonded to the metal has been shown to be characterized by two features:

1- It is essentially an enthalpy effect in that the decrease in log K_{ML} of metal – ligand complex is reflected mainly in a corresponding decrease in ΔH^O of complexation.

2- It is a composite effect, its magnitude depending upon the the elastic effect of the metallic cation on the ionizing group, as well as on the extent to which the stability of metal – ligand complex is enhanced by resonance.

The enthalpy changes of BIZ during the complex reaction seem always larger than those of BTZ and TZ complex formation. It is possible to say that the comparative exposure of one of the lmidazole nitrogen atom to solvent leads to the release of more solvent molecules on protonation, as tentatively suggested by Eilbeck et. al [56]

This work is concerned with the enthalpy of the formation of BIZ, BTZ and TZ complexes. In order to compare the thermodynamic properties of a series of BTZ, BIZ and TZ which have identical coordination sites and vary in their geometry.A similar reaction stereo chemistry with a series of metal ions UO_2(II), Pb (II), Ce (III), La (III), In(III), Zn (II), Cu (II) and Ni (II)was done. The enthalpies of formation of metal – ligand complexes in aqueous dioxane (30%, v/v) were determined, where the geometry of the reaction products are the same for a particular ligand. The differences between metal ions data are interpreted as a difference in the degree of solution of metal and ligand ions. By comparison, the enthalpy data of one ligand with those of another, difference in chelate geometry must be considered in accounting for the results Table (3.8-3.15).

Table (3-8): Thermodynamic stability constants and thermodynamic parameters for the formation of UO_2 (II) chelates of substituted azo dyes at(25 ± 0.1 C°, I = 0.1 M KNO_3 and 30%(v/v) 1,4 dioxane – water)

Azo dye no.	λ_{max}	Log K_{ML}	ΔG^o (-ve)	ΔH^o (-ve)	ΔS^o (+ve)	ΔHe (+ve)	ΔHc (-ve)
1	483[b]	8.37	47.8	13.5	115	11.5	25.0
2	490[b]	8.29	47.3	17.0	102	10.4	27.4
3	492[b]	8.49	48.5	19.1	99	10.2	29.3
4	487[s]	8.61	49.2	17.1	108	11.0	28.1
5	482[b]	8.73	49.8	13.6	121	12.0	25.6
6	497[b]	8.36	47.7	15.8	107	11.0	26.8
7	500[b]	8.19	46.8	10.9	120	12.0	22.9
8	483[s]	8.57	48.9	11.0	127	12.0	23.0
9	500[b]	8.48	48.4	9.8	129	13.0	22.8
10	493[b]	8.68	49.6	9.8	133	13.0	22.8
11	492[b]	8.50	48.5	11.9	123	12.1	24.0
12	500[s]	8.28	47.3	16.2	104	11.0	27.2
13	481[b]	8.60	49.1	19.6	99	10.2	29.8
14	489[b]	7.43	42.4	12.0	102	10.4	22.4
15	498[b]	8.41	48.0	17.1	104	11.0	28.1
16	490[s]	9.03	51.5	40.6	37	5.3	45.9
17	485[b]	8.68	49.6	41.7	26	4.4	46.1
18	480[s]	8.84	50.5	38.9	39	5.4	44.3
19	485[b]	9.34	53.3	44.5	29	4.8	39.7
20	562	4.37	24.9	19.1	19	3.9	23.0
21	568	4.50	25.7	17.8	26	4.4	22.2
22	570	4.48	25.6	17.3	28	4.6	21.9
23	550	4.60	26.3	22.8	12	3.3	26.1

Values of ΔG^o and ΔH^o in kJ / mole, ΔS^o in Joule / deg. mole and λ_{max}, maximum wave length at which the complex absorbed in nanometer unit .

Table (3-9): Thermodynamic stability constants and thermodynamic parameters for the formation of Pb (II) chelates of substituted azo dyes at(25 ± 0.1 C^o, I = 0.1 M KNO_3 and 30%(v/v) 1,4 dioxane – water) .

Azo dye no.	λ_{max}	Log K_{ML}	ΔG^o (-ve)	ΔH^o (-ve)	ΔS^o (+ve)	ΔHe (+ve)	ΔHc (-ve)
1	492[b]	7.76	44.3	6.7	126	12.3	19.0
2	485[s]	7.83	44.7	11.5	111	11.1	22.6
3	501[b]	7.86	44.9	1.4	146	13.9	15.3
4	480[b]	7.87	44.9	15.2	100	10.3	25.5
5	478[s]	7.87	44.9	6.1	130	12.6	18.7
6	486[s]	7.89	45.0	17.9	91	9.6	27.5
7	491[b]	7.80	44.5	16.0	96	10.0	26.0
8	480[b]	7.75	44.2	17.6	89	9.4	27.0
9	500[s]	7.85	44.8	9.9	117	11.6	21.5
10	495[b]	7.89	45.0	11.5	112	11.2	22.7
11	490[b]	7.78	44.4	6.8	126	12.3	19.1
12	489[b]	7.79	44.5	7.9	123	12.1	20.0
13	477	7.92	45.2	14.0	105	10.7	24.7
14	502[b]	7.70	44.0	6.4	126	12.3	18.7
15	503[s]	7.92	45.2	5.3	134	13.0	18.3
16	505[s]	8.02	45.8	43.1	9.0	3.1	46.2
17	510[s]	8.43	48.1	37.8	35	5.1	42.9
18	501[b]	8.20	46.8	40.2	22	4.1	44.3
19	505[b]	7.85	44.8	36.9	26	4.4	41.3
20	-	-	-	-	-		
21	-	-	-	-	-	-	-
22	550	3.00	17.1	11.3	19	3.9	15.2
23	500[b]	3.80	21.7	15.9	19	3.9	19.8

Values of ΔG^o and ΔH^o in kJ / mole, ΔS^o in Joule / deg. mole and λ_{max}, maximum wave length at which the complex absorbed in nanometer unit .

Table (3-10): Thermodynamic stability constants and thermodynamic parameters for the formation of Ce (III) chelates of substituted azo dyes at(25 ± 0.1 C^o, I = 0.1 M KNO_3 and 30%(v/v) 1,4 dioxane – water)

Azo dye no.	λ_{max}	Log K_{ML}	ΔG^o (-ve)	ΔH^o (-ve)	ΔS^o (+ve)	ΔHe (+ve)	ΔHc (-ve)
1	470[b]	8.68	49.6	20.0	99	10.2	30.2
2	477[b]	8.65	49.4	14.1	118	11.7	25.8
3	480[s]	9.02	51.5	3.5	161	15.1	18.6
4	475[b]	9.11	52.0	7.9	148	14.1	22.0
5	463[b]	9.09	51.9	12.6	132	12.8	25.4
6	477[b]	8.69	49.6	11.4	128	12.5	23.9
7	469[b]	8.62	49.2	7.3	141	13.5	20.8
8	460[b]	9.08	51.8	16.2	119	11.8	28.0
9	480	8.92	50.9	12.0	130	12.6	24.6
10	478[b]	9.02	51.5	16.0	119	11.8	27.8
11	476[s]	8.98	51.3	18.8	109	11.0	29.8
12	473	8.73	49.8	16.2	113	11.3	27.5
13	463	9.18	52.4	18.1	115	11.5	29.6
14	482[s]	8.87	50.6	19.4	105	10.7	30.1
15	478[b]	8.89	50.7	15.2	119	11.8	27.0
16	476[s]	10.68	61.0	50.7	35	5.1	55.8
17	47.6[s]	11.52	65.8	52.7	44	5.8	58.5
18	470[b]	11.30	64.5	43.3	71	8.0	51.3
19	472[b]	10.07	57.5	47.4	34	5.1	52.5
20	530[s]	6.36	36.3	31.7	15	3.5	35.2
21	535[s]	6.48	37.0	31.3	19	3.9	35.2
22	545[b]	6.66	38.0	28.5	32	4.9	33.4
23	522[b]	6.87	39.2	32.2	23	4.2	36.4

Values of ΔG^o and ΔH^o in kJ / mole, ΔS^o in Joule / deg. mole and λ_{max}, maximum wave length at which the complex absorbed in nanometer unit .

Table (3-11): Thermodynamic stability constants and thermodynamic parameters for the formation of La (III) chelates of substituted azo dyes at(25 ± 0.1 C^o, I = 0.1 M KNO_3 and 30%(v/v) 1,4 dioxane – water)

Azo dye no.	λ_{max}	Log K_{ML}	ΔG^o (-ve)	ΔH^o (-ve)	ΔS^o (+ve)	ΔHe (+ve)	ΔHc (-ve)
1	465^b	6.41	36.6	10.8	87	9.2	20.0
2	478	6.34	36.2	8.8	92	9.6	18.4
3	470^b	6.80	38.8	5.0	113	11.3	16.3
4	470^s	7.05	40.2	4.1	121	11.9	16.0
5	458^b	7.38	42.1	7.3	117	11.6	18.9
6	473^b	7.45	42.5	-0.2	143	13.7	13.5
7	478^b	6.08	34.7	-4.2	130	12.7	8.5
8	462^s	7.25	41.4	-1.6	144	13.8	12.2
9	480^b	6.74	38.5	-1.8	135	13.1	11.3
10	469^s	7.12	40.6	-8.1	163	15.3	7.2
11	474^b	6.62	37.8	2.0	120	11.9	13.9
12	469^s	6.21	35.5	3.5	107	10.8	14.3
13	463^b	7.34	41.9	11.1	103	10.5	21.6
14	470^b	7.60	43.4	-4.1	159	15.0	10.9
15	480^b	6.72	38.4	2.3	121	11.9	14.2
16	500^s	8.53	48.7	35.2	45	5.9	41.1
17	495^s	9.33	53.3	42.0	38	5.4	47.4
18	520^b	9.15	52.2	39.0	44	5.8	44.8
19	510^s	8.01	45.7	38.2	25	4.1	42.5
20	525^s	4.89	27.9	20.7	24	4.1	25.0
21	510^s	5.37	30.7	17.1	46	6.0	23.1
22	528^b	5.40	30.8	21.9	30	4.7	26.6
23	505^b	5.85	33.4	27.0	21	4.0	31.0

Values of ΔG^o and ΔH^o in kJ / mole, ΔS^o in Joule / deg. mole and λ_{max}, maximum wave length at which the complex absorbed in nanometer unit .

Table (3-12): Thermodynamic stability constants and thermodynamic parameters for the formation of In (III) chelates of substituted azo dyes at(25 ± 0.1 C°, I = 0.1 M KNO_3 and 30%(v/v) 1,4 dioxane – water)

Azo dye no.	λ_{max}	Log K_{ML}	ΔG^o (-ve)	ΔH^o (-ve)	ΔS^o (+ve)	ΔHe (+ve)	ΔHc (-ve)
1	488[b]	9.18	52.4	19.9	109	11.0	30.9
2	490[b]	9.14	52.2	27.0	85	9.1	36.1
3	493[s]	9.42	53.8	29.1	83	8.9	38.0
4	490[s]	9.55	54.5	30.7	80	8.7	39.4
5	483[s]	9.75	55.7	14.6	138	13.3	27.9
6	485[b]	9.52	54.3	24.9	99	10.2	35.1
7	492[b]	9.36	53.4	30.6	76	8.4	39.0
8	486[b]	9.23	52.7	28.6	81	8.8	37.4
9	496[b]	9.31	53.1	17.3	120	11.9	29.2
10	495[b]	9.50	54.2	21.3	110	11.1	32.4
11	488[b]	9.36	53.4	13.3	134	13.0	26.3
12	495[b]	9.04	51.6	14.1	126	12.3	26.4
13	480[b]	9.71	55.4	12.8	143	13.7	26.5
14	495[b]	9.26	52.9	6.0	157	14.8	20.8
15	493[b]	9.32	53.2	12.9	135	13.0	25.9
16	472[b]	11.62	66.3	54.3	40	5.5	59.8
17	463[s]	12.64	72.2	59.3	43	5.8	65.1
18	470[b]	12.20	69.6	48.3	71	8.0	56.3
19	465[b]	11.06	63.1	52.8	35	5.1	57.9
20	523[b]	5.38	30.7	24.1	22	4.1	28.2
21	527[s]	5.46	31.2	24.4	23	4.2	28.6
22	530[b]	5.68	32.4	21.6	36	5.2	26.8
23	505[b]	5.80	33.1	27.2	20	3.9	31.1

Values of ΔG^o and ΔH^o in kJ / mole, ΔS^o in Joule / deg. mole and λ_{max}, maximum wave length at which the complex absorbed in nanometer unit.

3.6 Biological Activity of the azo dyes: -

When the compound is synthesized. It was processed for an invitro anti-bacterial activity determination. Meantime, its observable minimum inhibitory conc(MIC)was determined. Table (3.17-3.18) in comparison to effect of chloramphenical, Gentamicin, Ampicilln and Tobramycin effect against standard, sensitive strains of *E.coli, Stap. aureus* and *Ps.aeroginosa* Table (3.16)

Inhibition zone of some well known antibiotic compounds using Kirby-Bauer method.

Antibiotic	Disc potency	Zone diameter of inhibition (mm)		
		E.coli	*Stap .aureus*	*Ps. aeroginosa*
Ampicilln	10 μg	16-22	27-35	----------
Chloramphenicol	30 μg	21-27	19-26	----------
Gentamicin	10 μg	19-26	19-27	16-21
Tobramycin	10 μg	18-26	19-29	19-25

Table (3-12) shows the zone diameter of inhibition of growth with tested organisms *E.coli, Stap. aureus* and *Ps. aergenosa*, towards the ligands and their complexes with Zn(II).

Stap. aureus was found invitro sensitive to these azo dyes derivatives .Azo –Zn II, combinations reduced such activity or stopping it. Thus antagonistic effect may be expectable and suggested. *E.coli* and *Ps. aergenosa* were found as invitro resistance to these azo dyes and azo derivatives. Thus, such compounds are invitro effective against *Stap. aureus*.

Table (3-13): Thermodynamic stability constants and thermodynamic parameters for the formation of Zn (II) chelates of substituted azo dyes at(25 ± 0.1 C^o, I = 0.1 M KNO_3 and 30%(v/v) 1,4 dioxane – water) .

Azo dye no.	λ_{max}	Log K_{ML}	ΔG^o (-ve)	ΔH^o (-ve)	ΔS^o (+ve)	ΔHe (+ve)	ΔHc (-ve)
1	510[b]	6.68	38.1	9.2	97	10.0	19.2
2	508[b]	6.60	37.7	3.7	114	11.4	15.1
3	518[b]	6.94	39.6	0.5	131	12.7	13.2
4	505[b]	7.23	41.3	-0.8	141	13.5	12.7
5	493[b]	7.43	42.4	11.4	104	10.6	22.0
6	512[b]	7.72	44.1	3.0	138	13.3	16.3
7	508[s]	6.50	37.1	4.9	108	10.9	15.8
8	498[s]	7.36	42.0	5.5	122	12.0	17.5
9	520[b]	6.92	39.5	6.3	111	11.1	17.4
10	505[b]	7.18	41.0	1.1	134	13.0	14.1
11	501[s]	6.88	39.3	1.2	128	12.5	13.7
12	506	6.49	37.0	5.5	106	10.7	16.2
13	493	7.27	41.5	0.5	138	13.3	13.8
14	505	7.80	44.5	5.6	130	12.6	18.2
15	517	6.93	39.6	3.6	121	11.9	15.5
16	510	6.78	38.7	23.1	52	6.5	29.6
17	527	7.05	40.2	25.2	50	6.3	31.5
18	520[s]	6.76	38.6	23.3	51	5.4	29.7
19	518[s]	6.55	37.4	21.8	52	6.5	28.3
20	-	-	-	-	-	-	-
21	-	-	-	-	-		
22	-	-	-	-	-		
23	490	3.74	21.4	14.1	24	4.3	18.4

Values of ΔG^o and ΔH^o in kJ / mole, ΔS^o in Joule / deg. mole and λ_{max}, maximum wave length at which the complex absorbed in nanometer unit .

Table (3-14): Thermodynamic stability constants and thermodynamic parameters for the formation of Cu (II) chelates of substituted azo dyes at(25 ± 0.1 C^o, I = 0.1 M KNO_3 and 30%(v/v) 1,4 dioxane – water) .

Azo dye no.	λ_{max}	Log K_{ML}	ΔG^o (-ve)	ΔH^o (-ve)	ΔS^o (+ve)	ΔHe (+ve)	ΔHc (-ve)
1	482[b]	8.20	46.8	17.1	100	10.3	27.4
2	478[b]	8.15	46.5	11.9	116	11.5	23.4
3	486[b]	8.32	47.5	7.2	135	13.0	20.2
4	468[b]	8.47	48.4	12.2	121	11.9	24.1
5	469[b]	7.75	44.2	12.6	106	10.7	23.3
6	480[b]	8.18	46.7	3.8	144	13.8	17.6
7	485[b]	8.05	46.0	12.5	112	11.2	23.7
8	467[s]	8.67	49.5	13.7	120	11.9	25.6
9	493[b]	8.26	47.2	6.2	138	13.3	19.5
10	486[b]	8.53	48.7	9.6	131	12.7	22.3
11	482[s]	8.31	47.4	11.9	119	11.8	23.7
12	485[b]	8.12	46.4	14.0	109	11.0	25.0
13	466[b]	8.63	49.3	14.2	118	11.7	25.9
14	470[s]	8.28	47.3	19.5	93	9.7	29.2
15	488[b]	8.20	46.8	6.80	134	13.0	19.8
16	472[s]	8.90	50.8	38.8	40	5.5	44.3
17	477[b]	9.27	52.9	37.1	53	6.6	43.7
18	480[s]	9.16	52.3	30.9	72	8.1	39.0
19	480	8.84	50.5	38.4	41	5.6	44.0
20	538	6.54	37.3	32.1	17	3.7	35.8
21	550	6.69	38.2	29.8	28	4.6	34.4
22	549	6.83	39.0	25.9	44	5.8	31.7
23	523	7.03	40.1	34.5	19	3.9	38.4

Values of ΔG^o and ΔH^o in kJ / mole, ΔS^o in Joule / deg. mole and λ_{max}, maximum wave length at which the complex absorbed in nanometer unit .

This investigation, using 15mg of each was impregnated on a disc, at physiological pH(7) and room temperature, shows that most of benzoimidazole, triazole and many benzothiozole derivatives have no antimicrobial activity, but there are some azo dyes, as indicated in Table (3-17) that behave to be active against one or more of the bacterial species tested. Different compounds have different anti-microbial activity, for this phenomena TableS (3-17-3-18) assigned that the compounds sample no. 1-4, 10-12, 22, 30-33 showed more bactericidal activity whereas others did not. Moreover, the activity of each compound showed a different pattern upon the tested organisms. This might be due to the change in the genetic properties of the living cells, *E. coli* was regarded as the most susceptible type of the three kinds of the tested organisms, where as the sample compounds showed previous degrees of sensitivity towards *Staph. aureus* and *Ps. Aerogenosa.* Most azo dyes and their complexes with Zn (II) showed a high affinity to gram +ve bacteria where as less extended against gram –ve (*Ps. aerogenosa*) except sample no.1. Samples 1-3, 10-12, 18 and 27-33 showed a remarkable inhibition zone diameters indicating a high activity in controlling the growth Fig. (3.27-3.33).

Table (3-15): Thermodynamic stability constants and thermodynamic parameters for the formation of Ni (II) chelates of substituted azo dyes at(25 ± 0.1 C°, I = 0.1 M KNO_3 and 30%(v/v) 1,4 dioxane – water) .

Azo dye no.	λ_{max}	Log K_{ML}	ΔG^o (-ve)	ΔH^o (-ve)	ΔS^o (+ve)	ΔHe (+ve)	ΔHc (-ve)
1	465[s]	7.78	44.4	5.9	129	12.6	18.5
2	465[b]	7.70	44.0	12.7	105	10.7	23.4
3	475[b]	7.85	44.8	6.6	128	12.5	19.1
4	470[b]	7.80	44.5	4.4	134	13.0	17.4
5	457[b]	7.83	44.7	14.9	100	10.3	25.2
6	468[s]	7.69	43.9	2.7	138	13.3	16.0
7	465[b]	7.73	44.1	5.6	129	12.6	18.2
8	457[b]	8.02	45.8	13.8	107	10.8	24.6
9	475[b]	7.77	44.4	7.3	124	12.2	19.5
10	473[b]	7.92	45.2	7.2	127	12.4	19.6
11	469[s]	7.75	44.2	0.2	148	14.1	14.3
12	463[b]	7.70	44.0	1.9	141	13.5	15.4
13	456[b]	7.90	45.1	7.3	127	12.4	19.7
14	472[b]	7.76	44.3	6.0	128	12.5	18.5
15	474[b]	7.87	44.9	10.4	116	11.5	21.9
16	471[b]	7.73	44.1	33.6	35	5.1	38.7
17	485[b]	7.95	45.4	32.7	43	5.8	38.5
18	787[s]	7.90	45.1	29.9	51	6.4	36.3
19	480[b]	7.61	43.4	32.9	35	5.1	38.0
20	493[b]	5.98	34.1	26.2	26	4.4	30.6
21	502	6.11	34.9	23.4	39	5.4	28.8
22	499	6.23	35.6	21.2	48	6.2	27.4
23	470	6.21	35.5	28.4	24	4.3	32.7

Values of ΔG^o and ΔH^o in kJ / mole, ΔS^o in Joule / deg. mole and λ_{max}, maximum wave length at which the complex absorbed in nanometer unit .

Fig. (3.27): The antimicrobial activity of sample no. 1, 2, 3, 5 & 6 show the inhibition of growth of *E. coli*

Fig. (3.28) The antimicrobial activity of sample no. 8, 9, 12, 15 & 16 show the inhibition of growth of *E. coli*

Fig. (3.29) The antimicrobial activity of sample no. 17, 18, 20, 21 & 23 show the inhibition of growth of *E. coli*

Fig. (3.30) The antimicrobial activity of sample no. 24, 26, 27, 35 &36 show the inhibition of growth of *E. coli*

Table (3-17) Inhibition zone of azo dyes and some of their derivatives (disk potency 14-15 Mg for each)

A/Compound	*E.coli*	*Stap.aureus*	*Ps.aeruginosa*
BTZ1	6	22	10
BTZ2	6	23	6
BTZ3	6	28	0
BTZ11	0	15	0
BTZ12	5	14	0
BIZ18	0	22	0
TZ20	0	18	0
TZ21	0	19	0
TZ22	0	24	0

Table (3-18) Inhibition zone of azo dyes and their complexes with Zn (II) (disk potency 14-15 Mg for each)

Ligand and ZnII	*Stap.aureus*
1- BTZ1	22
BTZ1+Zn	0
2- BTZ2	23
BTZ2+Zn	0
3- BTZ3	28
BTZ3+Zn	0
4- TZ 20	18
TZ+Zn	13
5- TZ 21	19
TZ21+Zn	12
6- TZ22	17
TZ22+Zn	5
7- TZ23	24
TZ23+ZN	6

Fig. (3.31) The antimicrobial activity of sample no.1, 2, 3, 11 &12 show the inhibition of growth of *Stap. aureus*

Fig. (3.32) The antimicrobial activity of sample no.22, 28, 29, 30 &31 show the inhibition of growth of *Stap. aureus*

Fig. (3.33) The antimicrobial activity of sample no.1, 2 show the inhibition of growth of *Ps. aerogenosa*

Fig. (3.27): The antimicrobial activity of sample no. 1, 2, 3, 5 & 6 show the inhibition of growth of *E. coli*

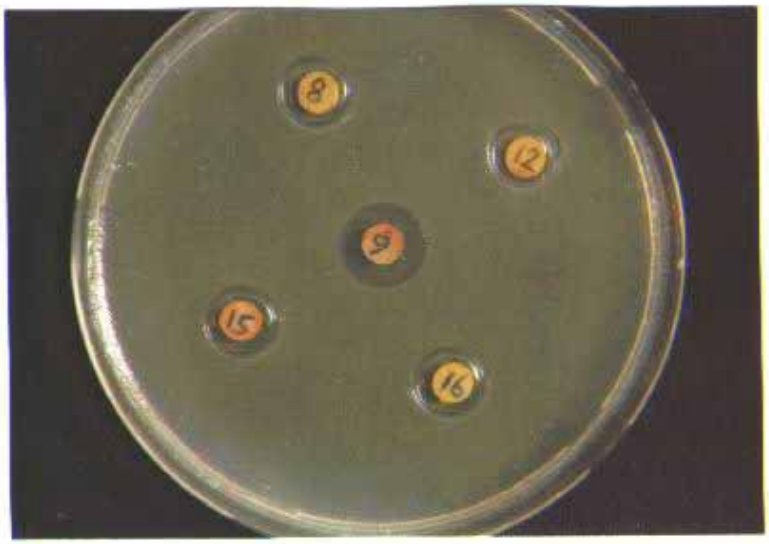


Fig. (3.28) The antimicrobial activity of sample no. 8, 9, 12, 15 & 16 show the inhibition of growth of *E. coli*

Fig. (3.29) The antimicrobial activity of sample no. 17, 18, 20, 21 & 23 show the inhibition of growth of *E. coli*

Fig. (3.30) The antimicrobial activity of sample no. 24, 26, 27, 35 &36 show the inhibition of growth of *E. coli*

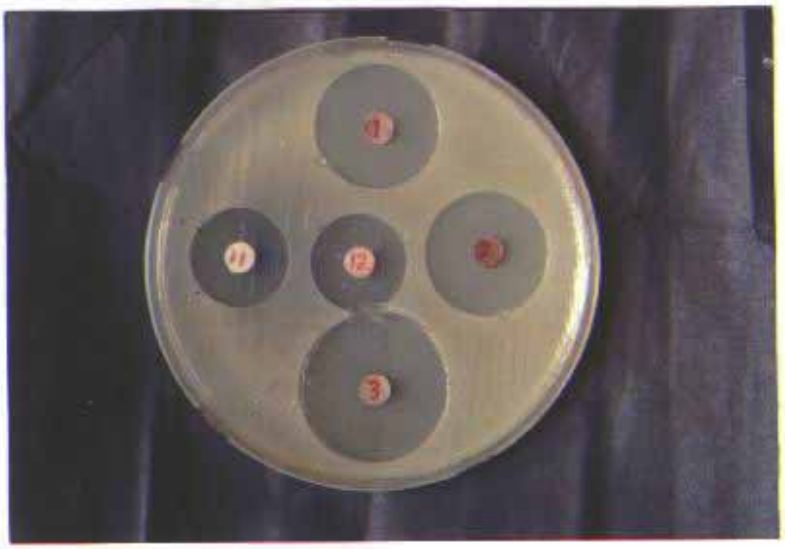


Fig. (3.31) The antimicrobial activity of sample no.1, 2, 3, 11 &12 show the inhibition of growth of *Stap. aureus*

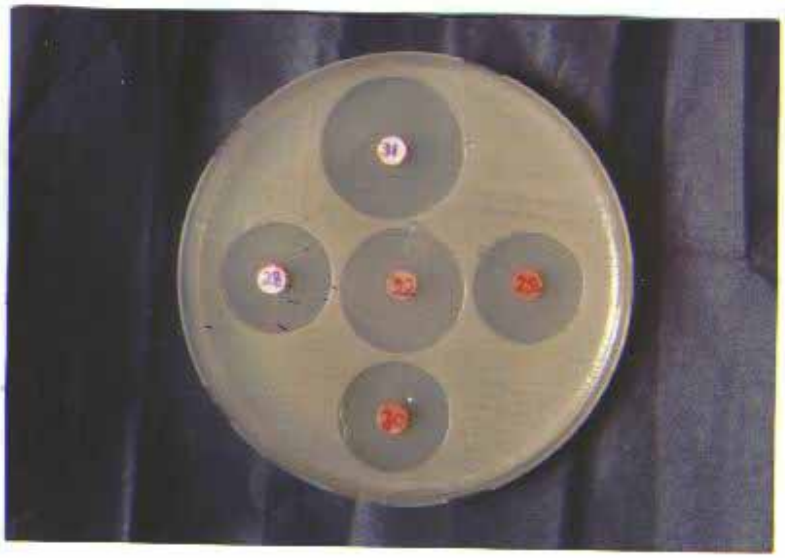


Fig. (3.32) The antimicrobial activity of sample no.22, 28, 29, 30 &31 show the inhibition of growth of *Stap. aureus*

Fig. (3.33) The antimicrobial activity of sample no.1, 2 show the inhibition of growth of *Ps. aerogenosa*

3.7 Conclusions:

1- The comparison between the three series of azo dyes, showed that the preparation of BTZ seems to be easier than those azo dyes of BIZ and TZ derivatives.

2- BTZ azo dyes are more acidic than BIZ compounds i.e pK_{a2} and pK_{a1} has lower values. While TZ ionization constants is equivalent to BTZ which might due to the presence of conjugation, the basicity of hydroxyl, azo group and a availability of electron around the donating groups, in addition to the presence of intramolecular hydrogen bonding.

3- λ_{max} shifts to the red region in the presence of electron donating group and to the blue shift in the case of with drawing group in the benzene ring of phenol while the substitution in the heterocyclic group show low extend.

4-Stability constants ($logK_{ML}$) of BIZ – complexes has higher values than the analogous BTZ while TZ-complexes seems the lowest ($logK_{ML}$) which might due to the basicity and the steriochemistry of ligands under consideration.

5-Decreasing of the ability of BTZ azo dyes compared with TAR to form complexes, so BIZ complexes comparing with PAR might due to basicity of ligands in addition to intramolecular hydrogen bonding.

6-The data in the work (i.e $\log K_{ML}$ and pK_{a2}) satisfied the linear relationship mainly:- $\log K_{ML} = \alpha\ pK_{a2} + \beta$. While some are not obey. The slope mostly less than unity, which explained due to the π-donation properties of metal ions under investigation.

7-BTZ azo dyes seem to react with metal ion as a tridentate ligand, bonded through the nitrogen atom of thiozole, the enolate nitrogen of azo group nearest to phenolic ring and oxygen of phenol group. In the same way BIZ and TZ azo dyes participated.

8-Thermodynamic parameters (ΔG^{θ}, ΔH^{θ} and ΔS^{θ}) referred that the reaction between ligands and metal ions, seems to be spontaneous nature, exothermic process and confirmed that the reaction formation were entropically favorable.

9-Entropy changes for BTZ-complexes seems to be more positive values than BIZ and TZ complexes, the highly value of (ΔS^{θ}) might due to the teutomerizem phenomena of BTZ during the reaction process.

10-The biological activities, in vitro showed that some of azo dyes were antibictirial agent against *E. coli*, *St . aureus* and *Ps. aerogenosa*, the results indicated that azo dyes were more effective against gram + ve bacteria and less extended to *Ps. aerogenosa*.

3.8 Further Scope: -

In the present investigation, a series of azo dyes were prepared and studied in a mixed solvent. It would be of interest to extend this work to study the effects of mole fraction of organic solvent on the ionization constants of these reagents and their reflection on the thermodynamic values of their complexes. Eight metal ions were chosen here one can extend it for other metal ions. Attempts should be made to synthesize various metal complexes in the solid state and study their structures, form and physical properties using different techniques available. It is desirable to confirm some of the structures by x – ray crystallography. In view of the reported applications of the azo dyes and their complexes a biological activity, analytical purposes, catalysis, organic synthesis and industrial applications.

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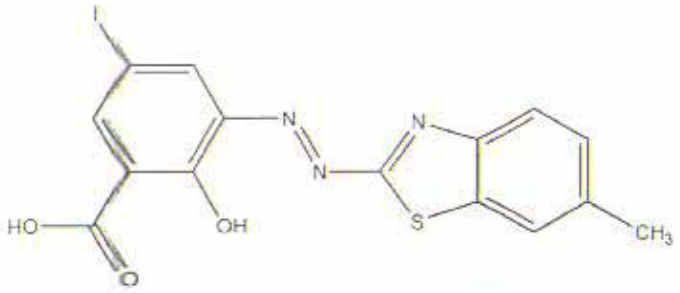
Appendix

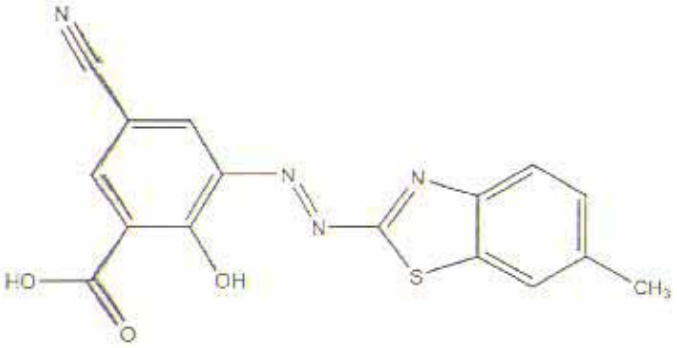
Azo-dye (1).



2-Hydroxy-5-iodo-3-(6-methyl-benzothiazol-2-Ylazo)-benzoic acid

$C_{15}H_{10}IN_3O_3S$
Exact Mass: 438.95
Mol. Wt.: 439.23
C, 41.02; H, 2.29; I, 28.89; N, 9.57; O, 10.93; S, 7.30

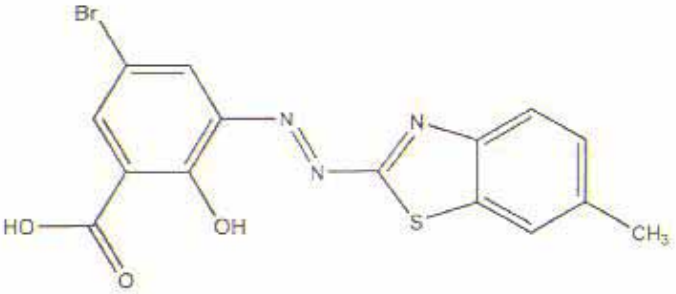
Azo-dye (2).



2-Hydroxy-5-Cyano-3-(6-methyl-benzothiazol-2-Ylazo)-benzoic acid

$C_{16}H_{10}N_4O_3S$
Exact Mass: 338.05
Mol. Wt.: 338.34
C, 56.80; H, 2.98; N, 16.56; O, 14.19; S, 9.48

Azo-dye (3).



2-Hydroxy-5-bromo-3-(6-methyl-benzothiazol-2-Ylazo)-benzoic acid

$C_{15}H_{10}BrN_3O_3S$
Exact Mass: 390.96
Mol. Wt.: 392.23
C, 45.93; H, 2.57; Br, 20.37; N, 10.71; O, 12.24; S, 8.18

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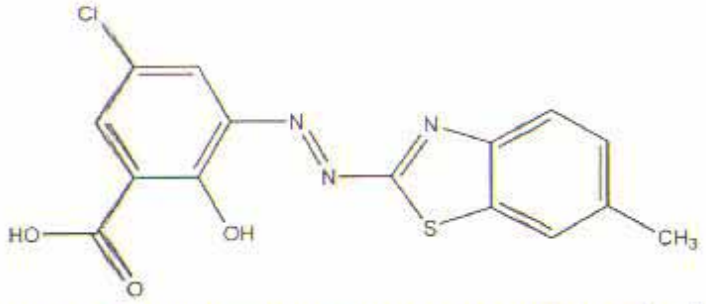
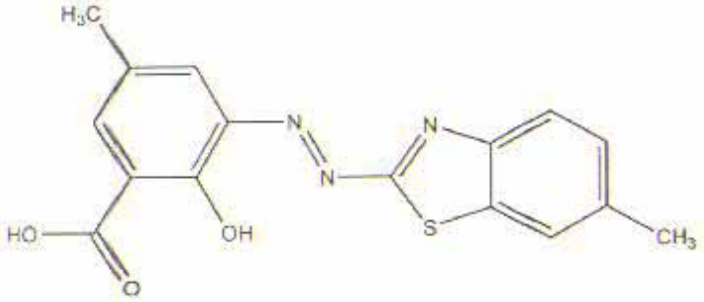
Azo-dye (4).



2-Hydroxy-5-chloro3-(6-methyl-benzothiazol-2-Ylazo)-benzoic acid

$C_{15}H_{10}ClN_3O_3S$
Exact Mass: 347.01
Mol. Wt.: 347.78
C, 51.80; H, 2.90; Cl, 10.19; N, 12.08; O, 13.80; S, 9.22

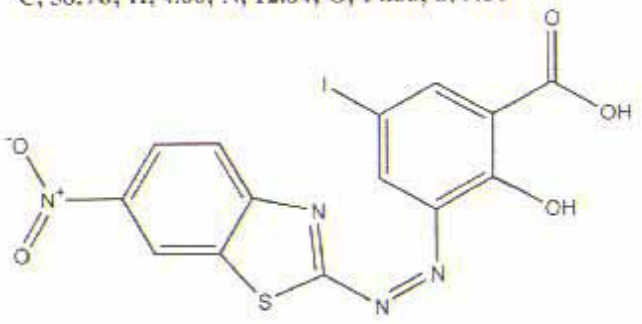
Azo-dye (5).



2-Hydroxy-5-methyl-3-(6-methyl-benzothiazol-2-ylazo)-benzoic acid

$C_{16}H_{13}N_3O_3S$
Exact Mass: 327.07
Mol. Wt.: 327.36
C, 58.70; H, 4.00; N, 12.84; O, 14.66; S, 9.80

Azo-dye (6).



2-Hydroxy-5-iodo-3-(6-nitro-benzothiazol-2-Ylazo)-benzoic acid

$C_{14}H_7IN_4O_5S$
Exact Mass: 469.92
Mol. Wt.: 470.20
C, 35.76; H, 1.50; I, 26.99; N, 11.92; O, 17.01; S, 6.82

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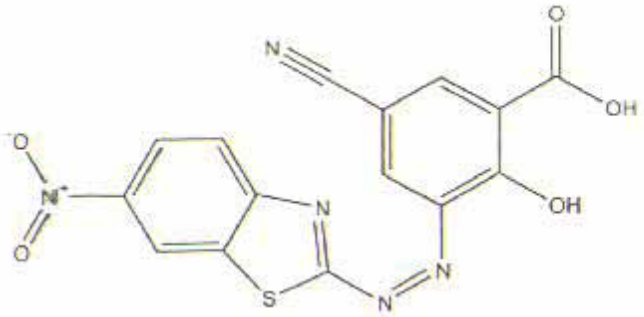
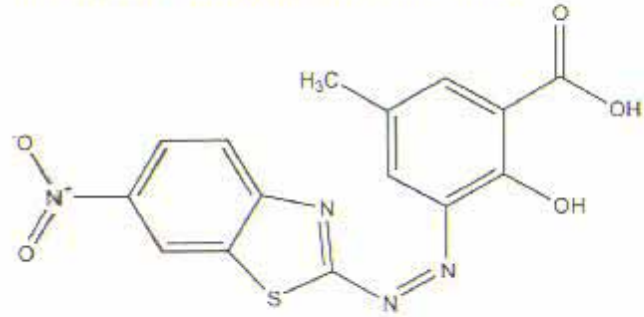
Azo-dye (7).



2-Hydroxy-5-cyano-3-(6-nitro-benzothiazol-2-Ylazo)-benzoic acid

$C_{15}H_7N_5O_5S$
Exact Mass: 369.02
Mol. Wt.: 369.31
C, 48.78; H, 1.91; N, 18.96; O, 21.66; S, 8.68

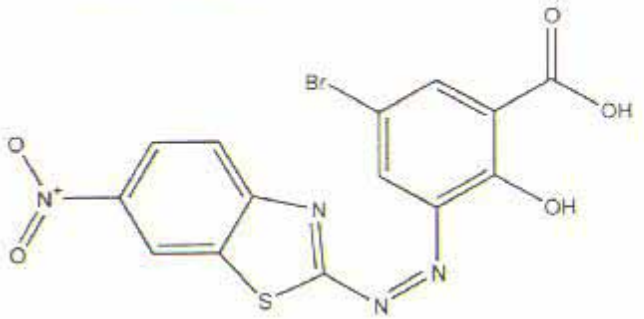
Azo-dye (8).



2-Hydroxy-5-methyl-3-(6-nitro-benzothiazol-2-Ylazo)-benzoic acid

$C_{15}H_{10}N_4O_5S$
Exact Mass: 358.04
Mol. Wt.: 358.33
C, 50.28; H, 2.81; N, 15.64; O, 22.32; S, 8.95

Azo-dye (9).



2-Hydroxy-5-bromo-3-(6-nitro-benzothiazol-2-Ylazo)-benzoic acid

$C_{14}H_7BrN_4O_5S$
Exact Mass: 421.93
Mol. Wt.: 423.20
C, 39.73; H, 1.67; Br, 18.88; N, 13.24; O, 18.90; S, 7.58

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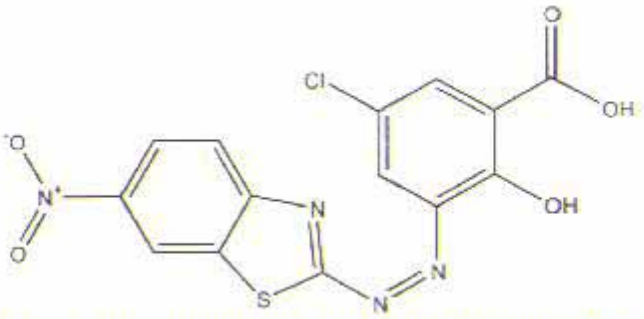
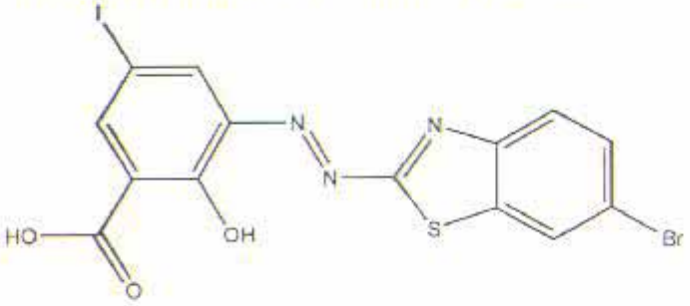
Azo-dye (10).



2-Hydroxy-5-chloro-3-(6-nitro-benzothiazol-2-Ylazo)-benzoic acid

$C_{14}H_7ClN_4O_5S$
Exact Mass: 377.98
Mol. Wt.: 378.75
C, 44.40; H, 1.86; Cl, 9.36; N, 14.79; O, 21.12; S, 8.47

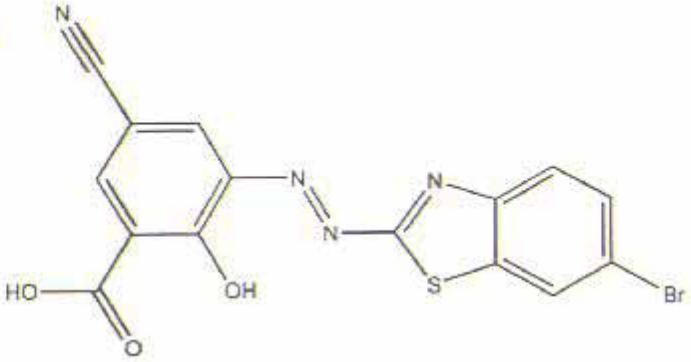
Azo-dye (11).



3-(6-Bromo-benzothiazol-2-ylazo)-2-hydroxy-5-iodo-benzoic acid

$C_{14}H_7BrIN_3O_3S$
Exact Mass: 502.84
Mol. Wt.: 504.10
C, 33.36; H, 1.40; Br, 15.85; I, 25.17; N, 8.34; O, 9.52; S, 6.36

Azo-dye (12).



3-(6-Bromo-benzothiazol-2-ylazo)-5-cyano-2-hydroxy-benzoic acid

$C_{15}H_7BrN_4O_3S$
Exact Mass: 401.94
Mol. Wt.: 403.21
C, 44.68; H, 1.75; Br, 19.82; N, 13.90; O, 11.90; S, 7.95

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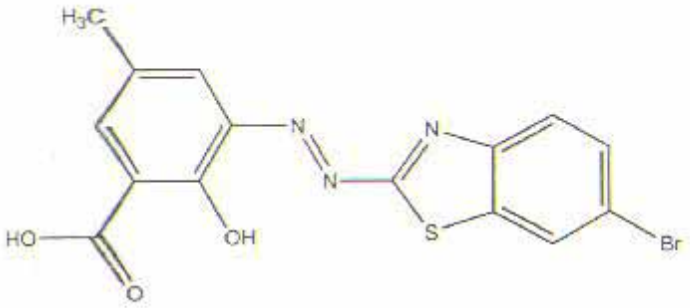
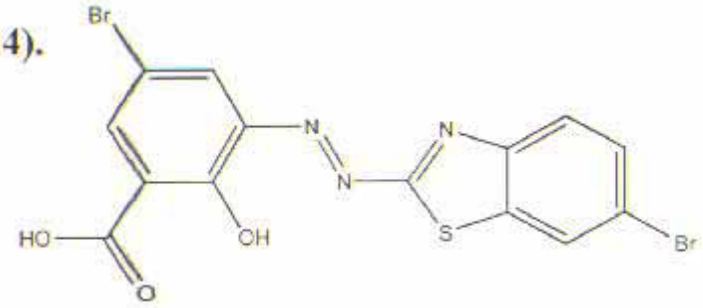
Azo-dye (13).



2-Hydroxy-5-methyl-3-(6-bromo-benzothiazol-2-Ylazo)-benzoic acid

$C_{15}H_{10}BrN_3O_3S$
Exact Mass: 390.96
Mol. Wt.: 392.23
C, 45.93; H, 2.57; Br, 20.37; N, 10.71; O, 12.24; S, 8.18

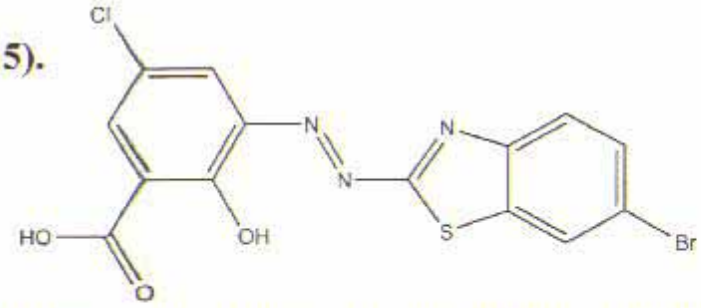
Azo-dye (14).



2-Hydroxy-5-bromo-3-(6-bromo-benzothiazol-2-Ylazo)-benzoic acid

$C_{14}H_7Br_2N_3O_3S$
Exact Mass: 454.86
Mol. Wt.: 457.10
C, 36.79; H, 1.54; Br, 34.96; N, 9.19; O, 10.50; S, 7.02

Azo-dye (15).



2-Hydroxy-5-chloro-3-(6-bromo-benzothiazol-2-Ylazo)-benzoic acid

$C_{14}H_7BrClN_3O_3S$
Exact Mass: 410.91
Mol. Wt.: 412.65
C, 40.75; H, 1.71; Br, 19.36; Cl, 8.59; N, 10.18; O, 11.63; S, 7.77

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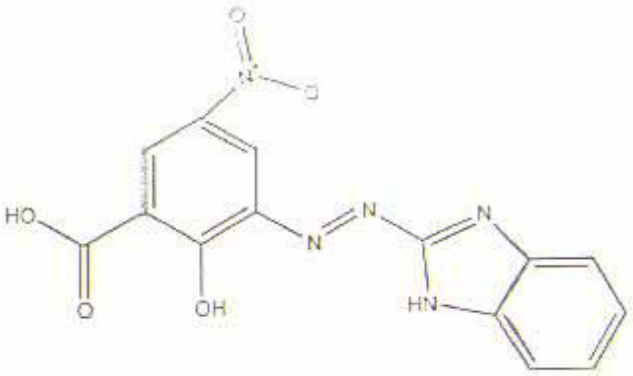
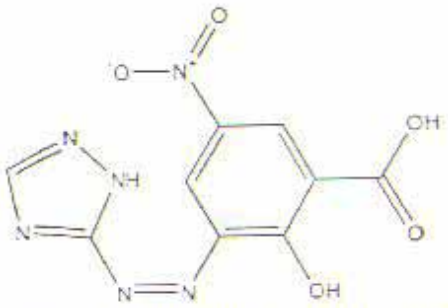
Azo-dye (19).



3-(1H-Benzoimidazol-2-ylazo5-nitro2-hydroxy-benzoic acid

$C_{14}H_9N_5O_5$
Exact Mass: 327.06
Mol. Wt.: 327.25
C, 51.38; H, 2.77; N, 21.40; O, 24.45

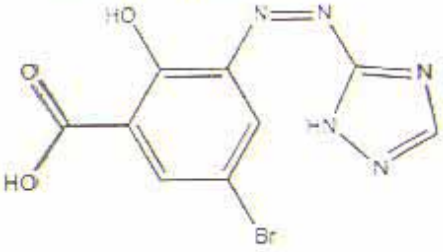
Azo-dye (20).



2-Hydroxy-5-nitro-3-(2H-[1,2,4]triazol-3-ylazo)-benzoic acid

$C_9H_6N_6O_5$
Exact Mass: 278.04
Mol. Wt.: 278.18
C, 38.86; H, 2.17; N, 30.21; O, 28.76

Azo-dye (21).



2-Hydroxy-5-bromo-3-(2H-[1,2,4]triazol-3-ylazo)-benzoic acid

$C_9H_6BrN_5O_3$
Exact Mass: 310.97
Mol. Wt.: 312.08
C, 34.64; H, 1.94; Br, 25.60; N, 22.44; O, 15.38

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وزارة التعليم العالي والبحث العلمي
جامعة بابـــــل
كليـــة العـــلوم
فســم الكيمـــــياء

تحضير ودراسة الخصائص والتاثير البايولوجي لعدد من اصباغ الازو الجديدة ومعقداتها مع عدد من الايونات الفلزية

رسالة مقدمة إلى
مجلس كلية العلوم / جامعة بابل وهي جزء من
متطلبات نيل درجة الدكتوراه فلسفة في علوم الكيمياء

تقدم ا
حسين عبد محمد صالح

ذي الحجة /١٤٢٣هـ اذار/ ٢٠٠٣م