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Extraction of Trace Metals in Natural Water Using Cyanidin/1-Butanol

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REG. NO: PG/M.Sc.-Ph.D/04/35772

A RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN INORGANIC CHEMISTRY IN THE DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY, UNIVERSITY OF NIGERIA, NSUKKA.

JULY, 2007.

APPROVAL PAGE

This research project has been approved for the Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, University of Nigeria, Nsukka.

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HEAD OF DEPARTMENT
DATE: 21/8/07

DR. C. O. B. OKOYE
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DATE: 21/8/07

EXTERNAL EXAMINER
DATE: 30/10/07

CERTIFICATION

MR. OKEKE LAZARUS CHILEE, a postgraduate student in the Department of Pure and Industrial Chemistry, and with Registration Number PG/M.Sc-Ph.D/04/35772, has satisfactorily completed the requirements for course and research work for the degree of Master of Science in Inorganic Chemistry. The work embodied in this project is original and has not been submitted in part or whole for any other diploma or degree of this or any other University.

DR. C.O.B. OKOYE
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DR. C. O. B. OKOYE
PROJECT SUPERVISOR
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DEDICATION

To Almighty God, for His provisions and sustenance and to my caring parents, MR. and MRS. FABIAN CHUKWUMA OKEKE, who said that I must take higher degrees and have been carrying the greater part of the burden.

ACKNOWLEDGEMENT

I wish to express sincere thanks to the following persons for the various ways they helped during the course of this research: to my able and resourceful research supervisor, Dr. C. O. B. Okoye for suggesting the topic and also supervising the work to completion; to Dr. P. O. Ukoha and Mr. J. N. Asegbeloyin for their advice, suggestions and criticisms.

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Finally, the assistance received from Mrs. N. N. Ukwueze and other staff of the Department is highly acknowledged.

ABSTRACT

The ligand 3, 3^1, 4^1, 5, 7-Pentahydroxyflavylium chloride was extracted in methanol/HCl (85:15 v/v) from the flowers of *Hibiscus rosa-sinensis*. The extraction process was inexpensive, efficient and fast. The UV/Visible spectra were also in agreement with those reported by past workers. Using methanol, as a solvent, the λmax was 532nm in the visible region, and 279nm in the ultraviolet region. The solubility of the crystals were tested in different organic solvents and found to be high in 1-butanol. Liquid-liquid extraction studies were also carried out on six heavy metal (Cd, Cu,Cr, Fe, Pb and Zn) complexes of 3, 3^1, 4^1, 5, 7-pentahydroxy flavylium chloride in 1-butanol as the organic solvent. The effect of pH on the extraction was also investigated. Complex formation with the heavy metals was confirmed from spectrophotometric analysis of both the free ligand and the complexed ligand with various metals in 1-butanol.The sensitivity of the extraction process was enhanced through pH adjustment which gave pH 7 as optimum. The mean percentage recoveries of the metals in tap water were: Cd (101.44), Cr (102.03), Pb (103.33), Fe (149.73), Zn (5.23) and Cu (117.94) while the mean recoveries in the river water, (in ppb) were; Cr (0.11), Cd (2.95), Zn (5.27), Pb (10.85), Cu (14.95) and Fe (30.81). Considering the cheapness and ready availability of this reagent over the conventional ligands, it could be very useful as an extractant for heavy metals recovery and concentration in natural waters.

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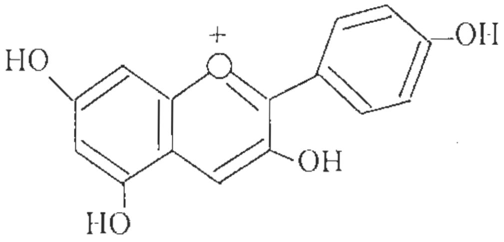
CHAPTER ONE
INTRODUCTION

1.1 Anthocyanins

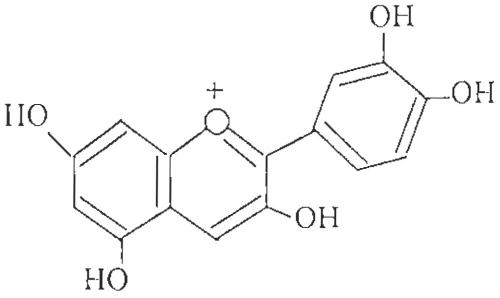
The anthocyanins are the most important and widespread group of colouring matters in plants [1]. These intensely coloured water-soluble pigments are responsible for nearly all the pink, scarlet, red, mauve, violet and blue colours in the petals, leaves and fruits of higher plants. The anthocyanins are all based chemically on a single aromatic structure, that of cyanidin [3, 3^1, 4^1, 5, 7-pentahydroxyflavylium ion], and all are derived from this pigment by addition or subtraction of hydroxyl groups or by methylation or glycosylation.

There are six commonly occurring anthocyanidins [2] [anthocyanin aglycones formed when anthocyanins are hydrolysed with acid]. These are pelargonidin, cyanidin, peonidin, delphinidin, malvidin, and petunidin (see Fig. 1-6). By far, the most common is the magenta-coloured cyanidin.



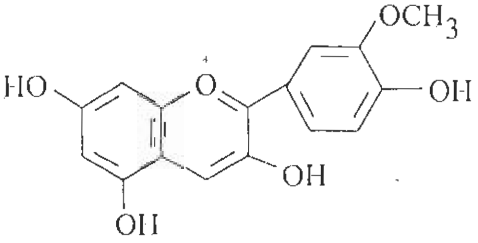
[1]

Pelargonidin



[2]

Cyanidin



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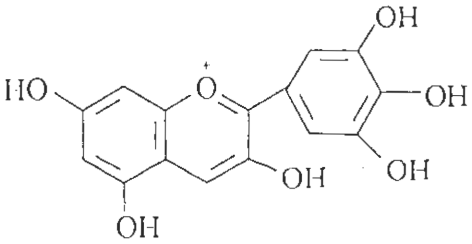
Peonidin



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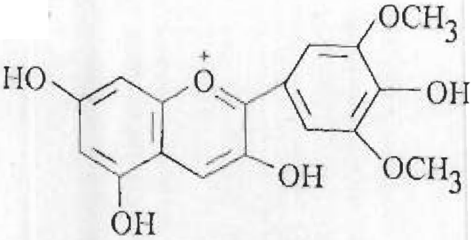
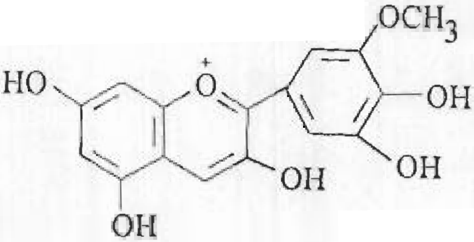
Delphinidin



[5]

Malvidin



[6]

Petunidin

Figures 1-6: Common Anthocyanidins

Each of the anthocyanidins occurs with various sugars attached forming a range of glycosides [i.e. as anthocyanins]. The main variation is in the nature of the sugar (often glucose, but may also be galactose, rhamnose, xylose or arabinose), the number of sugar units (mono, di – or tri – glycosides) and the position of attachment of sugar (usually to the 3-hydroxyl, or to the 3- and 5-hydroxyls) [1].

A significant number of anthocyanins occurs acylated with either an organic acid such as malonic or with an aromatic acid such as p-coumaric acid. Acylation is commonly through the sugar unit of the 3-position and both types of acylation may be present in the same molecule. Because of the diversity of glycosylation and acylation, there are at least 300 naturally occurring anthocyanins.

Anthocyanins, such as cyanin provide a natural sunscreen for plants. Because the molecular structure of an anthocyanin includes a sugar, production of this class of pigments is dependent on the availability of carbohydrates within a plant. Anthocyanin colour changes with pH, so soil acidity affects leaf colour. Anthocyanin production also requires light, so sunny days are needed for the brightest colours [3]. When leaves appear green it is because they contain an abundance of chlorophyll. Chlorophyll masks other pigment colours. Anthocyanins, in turn, mask carotenoids. As summer turns to autumn, decreasing light levels cause chlorophyll production to slow. However, the decomposition rate of chlorophyll remains constant, so the green colour will fade from the leaves. At the same time, anthocyanin production in leaves increases, in response to surging sugar concentrations. Leaves containing primarily anthocyanins will appear red. Leaves with good amounts of both anthocyanins and carotenoids will appear orange. Leaves with carotenoids but little or no anthocyanins will appear yellow. In the absence of these pigments, other plant chemicals can also affect colour [3]. An example includes tannins, which are responsible for the brownish colour of some oak leaves.

Solvent Extraction

Solvent Extraction involves the extraction of a solute molecule in an aqueous solution into a water-immiscible organic solvent. The interaction between a solute molecule and its neighbouring solvent molecules changes drastically when the solute molecule crosses the interphase of the two liquids. It can be summarized as involving the distribution of a solute between two immiscible phases. In this way, an extractive separation of the solute is effected.

For solvent extraction of metal chelates, metal ions are the solute in the aqueous phase, and are present in solvated form as metal-aquo complexes, metal ions being bonded to water molecules in the first or second sphere of attraction. The organic phase usually consists of the organic chelating agent dissolved in an organic solvent. For the metal to be extracted, the ionic complexes formed with water must be converted to neutral metal species called metal chelates, which distributes itself between the two liquid phases (in most cases completely soluble in the organic phase). Therefore, the efficiency of extraction among other factors depends to a large extent on the value of the partition coefficient and the number of extractions [4].

1.3 Environmental Impact of Heavy Metals

Metals are unique environmental and industrial pollutants in that they are found naturally distributed in all phases of the environment. However, through industrial processes they are concentrated and transformed into various products, and often this leads to human exposures to much higher concentrations or different chemical species than those naturally present in the environment [5].

Many metallic elements play vital biochemical roles in the maintenance of life. Some of them, example, sodium and iron are required in relatively large amounts while others such as manganese, copper, cobalt , molybdenum and zinc, are essential in trace amounts, but lethal or detrimental at higher concentrations. On the other hand, several others like lead, cadmium and mercury have never been known to have any biochemical functions to perform in any organism and have been found to be toxic even at low concentrations. Most heavy metals are toxic but the exact toxicity varies considerably. Toxicity also varies with chemical forms and the point of exposure. Few milligrams of methyl mercury chloride, CH_3HgCl, cause terrible ill effects while grams of mercury metal can be swallowed without serious effects. When metallic toxicants find their way into the body, they attack the proteins, notably the enzymes. The attack of heavy metals on the enzymes and proteins in cell membranes in the body interferes with the working order of the body system and the combined result of this attack leads to a variety of health problems ranging from cancer to heart diseases. In some cases, heavy metals may have either antagonistic or synergistic effects on the biological proportions of other metals. For example, selenium is antagonistic to mercury and reduces its toxicity. Also, some toxic metals are used in therapy for specific purposes despite the toxic risks[6].

All toxic heavy metals can endanger human health on slight exposure; the critical organs they affect in the body differ from metal to metal. The critical organ for Cd (II) and Hg (II) for example is the kidney, for CH_3Hg^+ it is the brain, lead affects the brain and hematological system and it is the most ubiquitous toxicants in the environment[7], while arsernic appears to be non-specific, it affects most organs or tissues but the skin is most significantly affected. Cadmium has a long biological life of twenty to thirty years in the kidney and chronic exposure may eventually accumulate to toxic levels, one of whose consequence may be Itai-Itai which is characterized by anemia, damaged proximal tubules, severe bone pain and mineral loss[8] . Lead and most of the other heavy metals have neurotoxic effects either on the peripheral nervous system (PNS) or on the central nervous system (CNS) and lead specifically, has been implicated in cot deaths and still-births[7]. A brief list of the neurotoxic effects of some of the heavy metals is given in the table below:

Table 1 Neurotoxicity of some of the heavy metals

Metals	Neurotoxic effects
Mercury	CNS: brain damage, visual, sensory, auditory and co-ordination malfunction. PNS: tremors i.e. involuntary agitations of the body or limbs due to physical weakness.
Lead	CNS: encephalitis (inflammation of the brain), inattention, IQ deficits. PNS: reduced nerve conduction
Arsenic	CNS: dizziness, restlessness, irritability, loss of hearing. PNS: motor paralysis, peripheral neuritis (nerve inflammation).
Indium	PNS: paralysis of limbs, convulsion movements.
Tellurium	PNS: tremors, diminished reflexes, convulsions.
Bismuth	PNS: peripheral neuritis
Selenium	CNS: depression, irritability.

The most often polluted of the environmental phases are the aquatic systems. This is because contaminants in the air, soil or on land ultimately end up in the aquatic system via local precipitation, water runoff and leaching of rocks and solid wastes [5]. Precipitation is an excellent collector and carrier of atmospheric pollutants such as gases [e.g. CO, SO_2, NO, NO_2], aerosol [e.g. $(NH_4)_2SO_4$] and particles [e.g. $CaCO_3$, Silicates] [8]. These are further transported into the aquatic systems by the runoff water. The most important pathway, by which aquatic systems receive wastes, is however by direct discharge of urban and industrial wastes. Experts estimate that urban and industrial wastewater introduce up to one million different potential pollutants into aquatic systems [9]. Nevertheless, a body of water is not regarded as polluted unless one or more of these various contaminants have rendered it unsuitable for an intended use, e.g., for domestic, industrial or agricultural water supply; propagation of fishery and wildlife; recreation; etc. [10]

1.4 Common Extracting Reagents for Metal Analysis

Extracting agents are weak acids and the number of elements they precipitate can usually be regulated by adjustment of the pH [13]. The reaction can be generalized as:

$$M^{n+} + nHX \longrightarrow MXn + nH^+$$

Where M^{n+} is the metal ion and nHX is the extracting reagent.

The weaker the metal chelate (MXn) formed, the higher the pH needed to achieve precipitation[14].

Extraction of metal complex from one solvent, usually water, into another often permits a considerable concentration of the complex to be achieved, with consequent increase in the effective sensitivity of an analytical procedure. It is also sometimes a very useful means of securing separations from interfering substances. Example of its analytical application include the extraction of thiocyanate complexes of Fe(III) and Mo(IV) from water by hydroxylic solvents, of Fe(III) from strong hydrochloric acid solutions by ethyl ether, of Cu(II) diethyldithiocarbamate into amylacetate, and of Ag, Hg, Cu, Pd, Bi, Zn, and Cd complexes of dithizone into chloroform or carbon tetrachloride. The solute may then be determined spectrophotometrically, or recovered quantitatively by evaporating the solvent or by back-extraction into an aqueous phase. This back-extraction may be made possible by the addition of oxidizing, reducing, or specific complexing substances, or by change of pH.

Solvent extraction reagents finding application in analytical chemistry include the following: tetraphenylarsonium chloride, quinaldic acid, triisooctylamine, o-phenanthroline, acetylacetone, benzoylacetone, bis-(acetylacetone)- ethylenediimine, salicyladoxime, bis-(salicylaldehyde)-o-phenylenediimine, bis-(trifluoroacetylacetone)-ethylenediimine, 8-hydroxyquinoline, 8-hydroxyquinaldine, dimethylglyoxime, thenoyltrifluoroacetone (TTA), 1-nitroso-2-naphthol, cupferron, dithizone, salicylic acid, etc. The properties of some extracting agents are given in the table below:

Table 2: Properties of Some Selected Chelating Agents used in Extraction[15]

Chelon	Molecular weight	Physical form at room temperature	pKa	K_D	Solvent
Acetylacetone	100.11	Colourless liquid	8.94	3.3 5.8 25	CCl_4 C_6H_6 $CHCl_3$
Benzyolacetone	162.18	White solid	8.7	660 1150 2500	CCl_4 C_6H_6 $CHCl_3$
thenoyltrifluoroacetone (TTA)	222.2	Yellow solid	6.23	40	C_6H_6
8-hydroxyquinoline	145.15	Colouless solid	4.91 9.81	206 400	$CHCl_3$ C_6H_6
8-hydroxyquinaldine	159.17	Colourless solid	5.77	6000	$CHCl_3$
Dimethylglyoxime	116.12	White solid	10.6	12.0 0.08	n-BuOH $CHCl_3$
1-nitroso-2-naphthol	173.16	Orange-brown solid	7.63	934 355	$CHCl_3$ MIBK
Cupferron	155.16	Buff solid	4.16	151 96.9	$CHCl_3$ MIBK
1-(2-pyridylazo)-2-naphthol (PAN)	248.26	Orange-red solid	2.9	10000	CCl_4
Dithizone	256.3	Violet-black solid	4.46	1.1×10^4 2.0×10^5	CCl_4 $CHCl_3$
Diethyldithiocarbamic acid	149.25	White solid	3.35	345 2360	CCl_4 $CHCl_3$
Salicylic acid	138.12	White solid	2.84	3 320	$CHCl_3$ MIBK

Equilibrium in solution between metal ions and complex-forming species are similar to those for protonation reactions. This is to be expected from the Lewis definitions of metal ions and protons as acids and of all ligands (because they are proton acceptors) as bases. Chelated complexes most often involving 5- or 6-membered ring formation, are usually more stable than the corresponding complexes involving monodentate ligands.[16] The few known exceptions can be explained in terms of steric strain, usually as a consequence of the preferred stereochemistry of the metal ion. Qualitatively, one would expect the chelate effect to become more important the more dilute the solution. This may be seen by comparing the complex behaviour of very dilute solutions of uni- and bidentate ligands. In the first case, attachment of a ligand to a metal ion does not enrich the solution around the 1:1 complex with ligand, whereas in the second case, attachment of one end of a bidentate ligand ensures the proximity of a second complexing group. Provided stereochemical requirements 'are met, the chelate effect increases with the number of chelate rings formed in the complex[16].

1.5 Aim of Project

The aim of the present research work is to

Extract cyanidin from one of its natural sources; *Hibiscus rosa-sinensis.*

Its application in metal recovery experiments from spiked tap water.

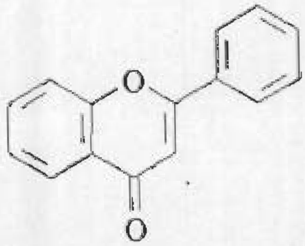
Study its application in the determination of some toxic heavy metals (Cd, Cu, Cr, Fe, Zn, and Pb) in water.

To assess its application in a one-step pre-concentration technique for heavy metal pollutional studies in natural waters.

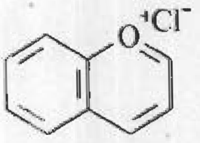
CHAPTER TWO
LITERATURE REVIEW

Chemistry of Anthocyanidins

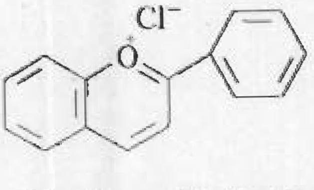
The various shades of colours exhibited by all flowers are due to a very small number of compounds that contain the same carbon skeleton but differ only in the nature of the substituent groups[17]. These natural pigments which exist as glycosides in aqueous cell sap were called anthocyanins and the sugar-free pigments – aglycones are known as anthocyanidins. Anthocyanins are widely distributed in plants and are responsible for the pink, red, purple and blue hues seen in many flowers, fruits and vegetables[18]. They are present in flowers, fruits and berries in amounts which vary from 0.4-24% of the dry substance. As a rule, the colour in a flower, fruit, or berry is due to only one anthocyanin[14]. Where mixtures exist, they are made up of methylated and unmethylated derivatives of the same anthocyanin. The anthocyanidins are a group of water-soluble flavonoid derivatives, which can be glycosylated and acylated and whose structure is based on the flavone (Fig. 7)[19]. Their fundamental nucleus is benzopyrillium chloride (Fig. 8) and their parent compound is 2-phenylbenzopyrillium chloride or flavylium chloride (Fig.9).



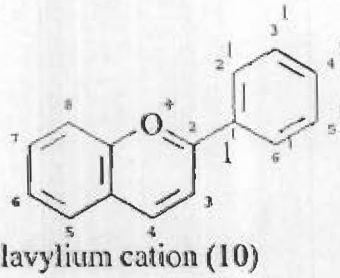
Flavone (7)



Benzopyrillium chloride (8)



Flavylium chloride (9)



Flavylium cation (10)

Some of the naturally occurring anthocyanins are summarized in Table 3 based on the structural unit of flavylium cation (Fig.10).

TABLE 3: Some Naturally Occurring Anthocyanidins [20].

Position of OH and H							Trivial Name	Chemical Name
	3	5	7	3^{I}	4^{I}	5^{I}		
1	OH	OH	OH	H	OH	H	Pelargonidin	3, 4^{I}, 5, 7-tetrahydroxyflavylium ion
2	OH	OH	OH	OH	OH	H	Cyanidin	3, 3^{I}, 4^{I}, 5, 7-pentahydroxyflavylium ion
3	OH	OH	OH	OCH_3	OH	H	Peonidin	3, 4^{I}, 5, 7-tetrahydroxy-3-methoxyflavylium ion
4	OH	OH	OCH_3	OCH_3	OH	H	Rosinidin	3, 4^{I}, 5-trihydroxy-3^{I}, 7-dimethoxyflavylium ion
5	OH	OH	OH	OH	OH	OH	Delphinidin	3, 3^{I}, 4^{I}, 5, 5^{I}, 7-hexahydroxyflavylium ion.
6	OH	OH	OH	OCH_3	OH	OH	Petunidin	3, 4^{I}, 5, 5^{I}, 7-pentahydroxy-3^{I}-methoxyflavylium ion
7	OH	OH	OH	OCH_3	OH	OCH_3	Malvidin	3, 4^{I}, 5, 7-tetrathydroxy-3^{I}, 5^{I}-dimethoxyflavylium
8	OH	OH	OCH_3	OCH_3	OH	OCH_3	Hirsutidin	3, 4^{I}, 5-trihydroxy-3^{I}, 5^{I}, 7-trimethoxyflavylium ion
9	H	OH	OH	H	OH	H	Apigenidin	4^{I}, 5, 7-trihydroxyflavylium ion
10	H	OH	OH	OH	OH	H	Luteolinidin	3^{I}, 4^{I}, 5,7-tetrahydroxyflavylium ion

Colour is their most important property because of its relationship with the appearance of flowers. The positions of the hydroxyl (OH) groups are the factors which control colour in the anthocyanidins and they have their greatest effect when present in the 2-phenyl group. An increase in the number of OH group causes a variation in colour from orange-red to blue-red in acid media[21]. Absorption spectra have been used for the quantitative estimation of anthocyanidin pigments in strawberries[22].

Extensive studies of 3, 3^{1}, 4^{1}, 5, 7-Pentahydroxyflavylium chloride have been carried out by many researchers. Willstatter and Everest isolated and identified cyanidin from cornflower. This was followed by the classical work on the isolation and analysis of the compound by Willstatters school. Harbone[23] and later workers[24] used both chromatography and spectrometry which are now universally accepted for the characterization and identification of the anthocyanidins.

The early use of 3, 3^1, 4^1, 5, 7-pentahydroxyflavylium chloride was as an acid-base indicator, although, modern synthetic indicators have relegated the use in this regard due to its poor bench strength. Recently, studies have shown that blackberry extract with cyanidin as its major constituent is a very useful drug in the treatment of cancer since it inhibits the growth of cancer tumour [25]. Bernard [26] discovered that compounds containing cyanidin chloride are useful for the treatment of antheroma and angiopatheies. Lietti [27] and co-workers also used this compound for treatment of wounds, stomach and duodenal ulcers, mouth and throat inflamations, pathological vascular disorder connected with lipids and glycerol metabolism because it inhibits low-density lipoproteins. It is an active component of bilberry (*vaccinium myrtillus*) which was used in the 12th century to induce menstruation and during World War II to improve British pilots' night vision (Retinopathy) [28,, 29]. It reduces stress-related parameter e.g. blood glucose level and arterial hypertension. It lessens minor bleeding, helps alleviate haemorrhoids and has a strong antioxidant [30] property because it is rich in vitamin C and E .

Anthocyanidins have found wide application also in food industries as red food colourants in countries like United States of America and Italy [26]. Thus, cranberry pigments have been used to enhance the colour of cherry pie filling. Anthocyanin extracts from red cabbage was reported to have a good colouring agent property when used for dry beverage mixes. Anthocyanidins of Saskatoon berry, a native of Southern Yukon and North West territories, the Canadian prariries and the Northern Prariries of USA was found to be important in the processing of important products such as jams, syrups, juices and jellies.

3, 3^1, 4^1, 5, 7-Pentahydroxyflavylium chloride can also be used as an analytical reagent. It was shown that aluminium chloride led to UV spectral shift of the anthocyanidins [23, 24]. The shift was attributed to complex formation between aluminium and the 3^1, 4^1 - 0 - dihydroxy system in the anthocyanidins [20]. Many workers like Maekawa et at [32] suggested that complex formation between Al (III) and anthocyanin was responsible for the blueing of petals in cut Chinese Bell-flower, *platycodon grandilorum*. Tin (II) which has a similar status as aluminium Chloride was reported to form stable coloured complex with cyanidin chloride [33].

2.1 Isolation, Identification and Characterization of Anthocyanidins

To isolate the anthocyanin, the flowers, fruits, or berry skins are extracted with either methyl alcoholic hydrogen chloride, acetic acid, or similar solvents. The salt is then usually precipitated as a syrup by the addition of large volumes of ether [19]. The precipitation process is repeated with variation of solvents, if necessary, until the anthocyanin is obtained in crystalline form as a hydrochloride or picrate. In some cases, the lead salt is used as an intermediate in the purification. To obtain an absolutely pure compound, the anthocyanin chloride or picrate may have to be re-crystallized for ten to thirty times [19]. For mixtures of anthocyanins, crystallization is not sufficient for separation, chromatography must be resorted to.

Hydrolysis of the anthocyanins with 20% hydrochloric acid gives anthocyanidins, which are still highly coloured, and either a sugar, a mixture of sugars, or a mixture of sugars and organic acids. The structure of the anthocyanidins has been determined by degradation with alkali and confirmed by synthesis. The structures of the anthocyanins and the points of attachment of the sugars have also been determined by oxidation, methylation studies, partition between immiscible solvents, colour reactions, comparison of physical properties, and synthesis. Melting points are accompanied by decomposition and are of no value for identification purposes. Such studies divide the anthocyanins into the following five classes;

3-Monoglucosides, 3-Monogalactosides, and 3-Monopentosides.
3-Rhamnoglucosides and other 3-Pentose glycosides.
3-Biosides
3, 5-Diglucosides
Complex anthocyanins or acylated derivatives of the above classes in which acids such as malonic, p-hydroxycinnamic, and p-hydroxybenzoic are involved.

The partition of anthocyanins between immiscible solvents is useful for the identification, separation and purification of anthocyanins. In identification work, the partitioning is represented as a distribution number, or the percent of anthocyanin extracted by the organic solvent from the aqueous medium under definite conditions. Isoamyl alcohol and 0.5% hydrochloric acid are best suited for monoglucosides, and 0.5% hydrochloric acid and n-butyl alcohol are preferred for diglucosides since these are too insoluble in amyl alcohol. The amount of anthocyanin in the organic layer is measured calorimetrically.

The distribution constants for pure anthocyanins are mainly dependent on the sugar residues present and are increased markedly by the introduction of acyl groups into the sugars as in the complex anthocyanins [19], and decreased only slightly by reducing the number of hydroxyl groups by one. The method is superior to the application of absorption spectra and colour reactions for identification purposes in cases where the main variation is the sugar.

The partition method can be used to separate anthocyanidin from anthocyanins, since the former are soluble quantitatively in the amyl alcohol whereas the anthocyanin remains mainly in the 0.5% hydrochloric acid. Other methods of identification are dependent upon comparison of outward physical properties, such as crystalline form, appearance in the solid and molten states, solubility, fluorescence, and colour in solutions of different pH.

2.1.1 Use of Colour Reactions for Identification Purposes

The colour reactions and partition behaviours of pure anthocyanins have been developed into rapid qualitative tests which can be used to determine the type of anthocyanins present in plants. Best results are obtained with these by examining a cold 1% aqueous hydrochloric acid extract of the fresh flowers as soon as possible after preparation.

The tests are as follows[19]

Cyanidin Reagent: A portion of the cold 1% aqueous hydrochloric acid extract of the fresh flowers is shaken with an equal volume of a mixture of cyclohexanol (1 vol.) and toluene (5 vol.).

Delphinidin Reagent: A portion is shaken with a 5% solution of picric acid in a mixture of amyl ethyl ether (1 vol.) and anisole (4vol.).

Oxidation Test: A portion is shaken with air and half its volume of 10% sodium hydroxide is added. This is immediately followed by concentrated hydrochloric acid and amyl alcohol to determine whether the anthocyanidin can be recovered.

Colour Test: To a portion extracted with amyl alcohol, sodium acetate is added and the colour is observed. This is followed by the addition of a drop of ferric chloride with shaking and the colour again observed.

The results observed with various anthocyanidins are given in Table 4.

Reagent	Pelargonidin	Cyanidin	Malvidin	Petunidin	Delphinidin
Cyanidin reagent	largely extracted	Rose colour	No extraction	No extraction	No extraction
Delphinidin reagent	Completely Extracted	Not completely extracted	Completely extracted	No extraction	No extraction
Oxidation	Not destroyed	Fairly stable	Unchanged	Destroyed	Destroyed
Acid solution	Red	Violet-red	Violet-red	Violet-red	Bluish-red
Amyl alcohol-NaOAc	Violet-red	Reddish-violet	Blue-violet	Violet-blue	Blue
$FeCl_3$	Violet-red	Bright blue	Blue-violet	Pure blue	Blue

2.1.2 Spectral Methods of Identification

Anthocyanidin has been analysed by GLC – MS (gas-liquid chromatography and mass spectroscopy) [34]. Trimethylchlorosilane [TMCS] is used to derivatise the anthocyanidin and consequently analysed by GLC – MS.

Recently, negative Nuclear Over Hauser's Effect (NOE) method has been used in elucidating the structure of anthocyanin from the morning glory *IPomoea tricolor*. The anthocyanidin found present was peonidin [35].

The absorption spectra of a large number of chromatographically pure pigments were measured by Harborne [22]. From his results, it was apparent that this method could be used to characterize anthocyanins and anthocyanidins.

Examinations are usually done in MeOH/0.01%HCl to avoid variations arising from the amphoteric nature of the pigments. It was observed that addition of $AlCl_3$ to an anthocyanindin produces a large bathochromic shift only when the 3^1 and 4^1-dihydroxy groups are present.

Frequently, it is desirable to express anthocyanin determinations in terms that can be compared with the results from different workers. The best way to express these results is in terms of absolute quantities of anthocyanins present [36].

The total anthocyanin content in crude extracts containing other phenolic materials has been determined by measuring absorptivity of the solution at a single wavelength. This is possible because anthocyanins have a special absorption band in the 490 to 550nm is possible because anthocyanins have a special absorption band in the 490 to 550nm region of the visible spectra. This band is far from the absorption bands of other phenolics, which have spectra maxima in the UV range [36]. In many instances, however, this simple method is inappropriate because of interference from anthocyanin degradation products or melanoidins from browning reactions [37]. In those cases, the approach has been to use differential and/or subtractive methods to quantify anthocyanins and their degradation products.

The differential method measures the absorbance at two different pH values, and relies on the structural transformations of the anthocyanin chromophore as a function of pH. Fuleki and Francis used pH 1.0 and 4.5 buffers to measure anthocyanin content in cranberries, and modifications of this technique have been applied to a wide range of commodities [38].

Subtractive methods are based on the use of bleaching agents that will decolour anthocyanins but not affect interfering materials. A measurement of the absorbance at the visible maximum is obtained, followed by bleaching and remeasuring to give a blank reading. The two most used bleaching agents are sodium sulfite and hydrogen peroxide [39].

By using both of these spectral procedures, accurate measurement of the total monomeric anthocyanin pigment content can be obtained, along with indices for polymeric colour, colour density, browning and degradation. The bisulfite bleaching reaction is utilized to generate the various degradation indices. While monomeric anthocyanins are readily bleached by bisulfite at product pH, the polymeric anthocyanin-tannin and melanoidin pigments are resistant and will remain coloured. Absorbance measurements are taken at the $\lambda_{\text{vis-max}}$ and at 420nm on the bisulfite bleached and control samples. Colour density is the sum of the absorbances at the $\lambda_{\text{vis-max}}$ and at 420nm of the control sample, while polymeric colour is the same measurement for the bisulfite treated sample. A measure of percent polymeric colour is obtained as the ratio between these two indices. The absorbance at 420nm of the bisulfite-treated sample is an index for browning, as the accumulation of brownish degradation products increases the absorption in the 400 to 440nm range. The absorption of these compounds is in general not affected by the addition of a bisulfite solution.

Substantial information can be obtained from the spectral characteristics of anthocyanins. Two distinctive bands of absorption, one in the Uv-region (260 to 280nm) and another in the visible region (490 to 550nm) are shown by all anthocyanins. The different aglycons have different $\lambda_{\text{vis-max}}$, ranging from 520nm for pelargonidin to 546nm for delphinidin, and their monoglucosides exhibit their $\lambda_{\text{vis-max}}$ at about 10 to 15 nm lower [40]. The shape of the spectrum may give information regarding the number and position of glycosidic substitutions and number of cinnamic acid acylations. The presence of cinnamic acid acylation is revealed by the presence of a third absorption band in the 310 to 360nm range, and the ratio of absorbance at 310 to 360nm to the absorbance at the visible $\lambda_{\text{vis-max}}$ will give an estimation for the number of acylating groups [41]. The solvent used for spectral determination will affect the position of the absorption bands, and therefore must be taken into consideration when comparing available data.

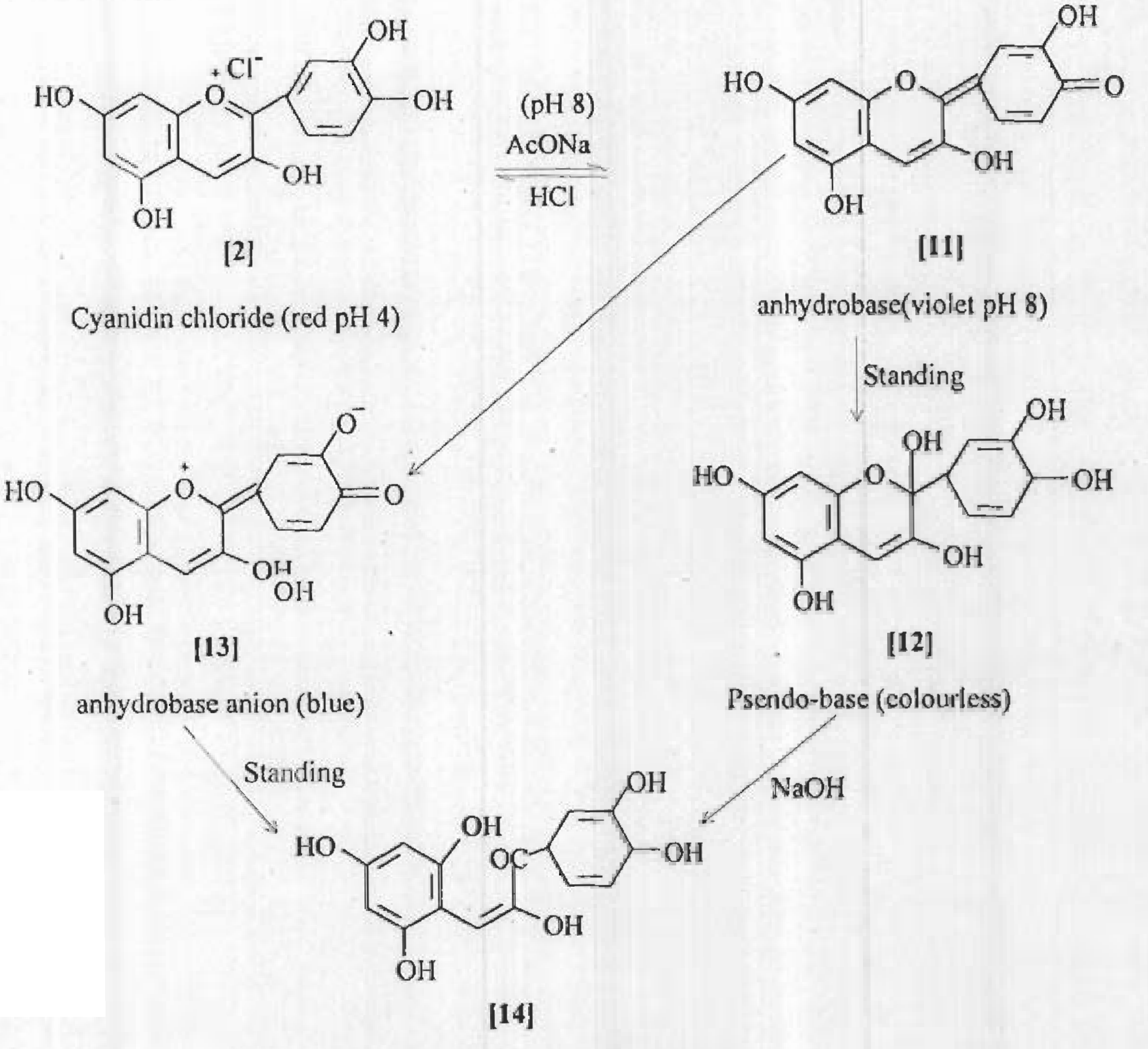
2.2 Cyanidin

Cyanin diglucoside has been identified as the anthocyanin present in *Hibiscus rosa-sinensis* flowers and blackberry, with cyanidin as the aglycone or anthocyanidin. *Hibiscus rosa – sinensis* is the best known of all the species of Hibiscus flowers. It has flowers which have slightly reflexed petals, a dark spot in the centre and a long red staminal column. The colours range from orange to the darkest of shades. Its height varies from 3 – 12 feet.

Cyanidin is one of the four most common anthocyanidins [34]. Physically, it is a reddish coloured crystalline substance, insoluble in water but soluble in organic solvents such as acetone, methanol and ethanol. Cyanidin like other anthocyanidins lack sharp melting point and is unstable at alkaline pH giving blue to green compounds [42].

When cyanidin chloride is kept in an aqueous sodium acetate solution (pH 8), the solution is violet due to the formation of the anhydrobase. The solution becomes colourless on standing due to loss of quinonoid structure of the anhydrobase and a pseudo-base is formed. When the pH is raised, that is, made alkaline by the addition of NaOH (pH 12), an anion of the anhydrobase which is blue is formed. When this solution is made acidic (pH 4); the reddish colour of cyanidin chloride is regenerated. On the other hand, on standing (in pH 12) in alkaline solution, all of these compounds are converted into the yellow chalcone [20].

Various forms of 3, 3^{1}, 4^{1}, 5, 7-Pentahydroxy flavylium chloride in varying pH conditions.



[2]

Cyanidin chloride (red pH 4)

[11]

anhydrobase(violet pH 8)

[13]

anhydrobase anion (blue)

[12]

Psendo-base (colourless)

[14]

Chalcone (yellow, pH 12 and above)

2.3 Classification of Metals

Metals have been classified by wood [43] from the stand point of environmental pollution, toxicity and bioavailability. According to him, metals are grouped as follows:

non – toxic
toxic but very insoluble or very rare; and
very toxic and relatively accessible.

Among the very toxic and bioavailable ones are Ti, V, Cr, Mn, Co, Ni, Cu, As, Cd, Ag, In, Sn, Sb, Pb, Hg and Bi.

In the same regard, Evert and Richardson [44] while trying to explain the chemistry of heavy metal toxicity, arranged the metals in three classes as follows: class A (oxygen – seeking), class B (nitrogen/sulphur seeking) and borderline class. While metals of classes

A and B are ligand-selective, the metals in borderline class can form complexes with all categories of ligands. Among the borderline metals are Ga^{3+}, Cd^{2+}, In^{2+}, Sn^{4+}, Pb^{2+}, As (III), Sb (III) and all members of the first row of the transition metals in the periodic table.

On the basis of atomic number and specific gravity, heavy metals have been defined as those whose atomic numbers are 22 – 34, 40 – 52, in addition to the lanthanides and the actinides or those metals having specific gravities greater than 4 g/cm^3 [45]. Most heavy metals are therefore toxic and belong to either class B or the borderline class.

Pearson [46] also classified metals and ligands into the "hard and soft" categories on the criteria of electron mobility or polarizability and electronegativity. He explained the "Hard and soft Acids and Bases" theory by saying that hard acids such as Mg^{2+}, Ca^{2+} and Al^{3+} form very stable complexes with hard bases such as O^{2-} and $CO^{2-}{}_3$, while the soft acids like Hg^{2+} prefer the soft bases such as S^{2-} and NO_3^- to form stable complexes.

2.3.1 Sources of Heavy Metals

Heavy metals are present at much lower concentrations in waters compared to major ions [SO_4^{2-}, Cl^-, NO_3^-, Mg^{2+}, Ca^{2+}, etc] hence they are commonly referred to as 'trace metals' [47].

Major anthropogenic sources of heavy metals are industrial wastes from mining, manufacturing and metal finishing plants. Other anthropogenic sources of metals in surface waters include domestic waste waters and runoff from roads. Metals are cycled through the environment and they may enter surface waters in precipitation. Therefore, metals emitted into the atmosphere by industrial processes (e.g. Hg from the combustion of coal, Pb from petrol) end up in surface or groundwaters.

They may also be leached from soils and rocks in contact with water. The disposal of massive quantities of metal wastes at land fills can lead to metal pollution of ground and surface waters. Acidification of surface and ground waters by acid rain could lead to increased leaching of metals and hence higher concentrations of trace metals in drinking water owing to the acid-induced leaching of metals from pipes.

Many of the trace metals are highly toxic to humans [e.g. Ag, Pb, Cd, Ni, As, Sn] and other living organisms, and their presence in surface waters at above background concentrations is undesirable. Also, unlike many organic pollutants, metals cannot be degraded in the environment by chemical or biological processes. They can, however, react in the environment, but the resulting metal-containing compounds may be even more toxic [e.g. methylation of Hg]. Toxic metals may also be 'biocentrated' in the food chain so that concentrations in the upper members of the chain can reach values many times higher than in water. The result is that some plants and animals may present a serious hazard if consumed as food. Toxic metal pollution has been identified in surface and coastal waters throughout the world and it is becoming a major environmental problem. Usually, treatment at source is the only practical way to control toxic metal pollution.

The concentrations of some heavy metals in different waters are shown in Table 5 below:

Table 5 concentrations of some heavy metals in water samples (μg/L) [47].

Metal	Seawater	River water	Rain water	Tap water
Cd	-	0.1 – 20	-	< 2
Cr	-	0.5 – 100	-	< 4
Co	-	1 – 50	-	< 5
Cu	5	1 – 300	0.1 – 150	15 – 25
Fe	50	1 – 5000	1 – 300	30 – 100
Pb	-	0.2 – 150	0.02 – 50	3 – 10
Mn	5	0.3 – 3000	0.3 – 30	5 – 20
Hg	0.03	0.1 – 0.2	-	-
Ni	0.1	1 – 150	0.1 – 100	< 5
Ag	0.3	0.1 – 30	-	< 3
V	0.3	2 – 300	-	5 – 15
Zn	5	2 – 1200	0.2 – 150	10 - 20

Seawater has very low concentrations; however, coastal waters receiving sewage and other effluents can have considerably higher concentrations than those shown in the table. Concentrations in river waters can vary considerably. Background concentrations in remote areas may be below the range shown in the table, while highly polluted waters in industrial zones may have even higher levels. Rainwater concentrations are generally lower than those observed in river water. Concentrations of some metals [Zn, Pb, Cu] in first-draw tap water can be considerably higher than those shown in the table owing to the accumulation of metals leached from pipes in standing water.

2.4 Techniques for Trace Metal Analysis

To procure analytical data of significance, two important preliminary steps must be taken prior to an analysis. These are
(i) Sampling and (ii) Sample preparation [4]. The two steps are aimed at producing a homogenous mixture to be analysed. For trace metal analysis, the exact nature of the procedures, of course depends on:
(i) The aim of the analysis,
(ii) The nature of the samples,
(iii) The elements to be determined and the analytical concentrations expected, and
(iv) The detection technique available.

The reliability of the analytical results are significantly influenced by the manner in which the samples are collected, preserved, stored, or otherwise processed, prior to analysis and often, the detection technique is decisive on the method of sample preparation to be employed [5].

The most widely used technique for determination of trace metals in environmental samples is the atomic absorption spectrophotometry. Other techniques that have been used are x-ray fluorescence spectroscopy [48], neutron activation analysis [49], anodic stripping voltametry [50] and inductively coupled plasma emission spectroscopy [51]. Each of these techniques requires one form or another of preliminary sample treatment before reliable determinations of the trace metals can be made.

Atomic absorption spectrophotometry with flame atomization (flame-AAS) requires that the sample be in solutions. The sample aerosol is produced by a pneumatic nebulizer system and introduced into the flame where the solvent is evaporated, and the sample vapourized and atomized to produce ground state atoms. These ground state atoms then absorb characteristic wavelength from a hollow cathode lamp excitation source.

Apart from the use of flame, vapour techniques and electrically heated non-flame atomizers are also used. The electrothermal devices include the graphite tube furnace and the graphite rod. However, these have limited use. For example, vapour techniques are limited to a few volatile metals such as Hg, Sb, As and Bi. With electrothermal atomization, solids can be analysed directly.

Another highly sensitive technique which can be used directly to measure concentrations of trace metals in natural water is the Anodic stripping voltametry (ASV).

It is most useful in speciation studies in distinguishing between "labile" and "bound" metal species [50]. The labile species are the free metal ions plus metal complexes which will dissociate in the diffusion layer to liberate the metal ion. Bound metal is a metal combined in relatively inert complexes and is defined as total minus labile metal. Total metal can be determined after irradiation with u.v. light. Preliminary chemical separations such as by liquid-liquid extraction, dialysis, elecrophoresis, ultrafiltration and centrifugation can be applied to achieve better results.

Anodic stripping voltametry is a two-step process of concentration and analysis in an electrolytic cell. The electroactive metals in solution in the presence of large excess of a support electrolyte, e.g., KCl, are deposited on a hanging mercury drop cathode when the potential made 0.2 – 0.4 V more negative than the highest reduction potential of the reducible ions. After electrodeposition, for a relatively very short time (0.25 – 0.65 sec), the connections to the cell are reversed, and a gradually increasing potential is applied to the hanging mercury drop, which is now the anode of the cell. Peak current due to the oxidation of any of the metals in the mercury amalgam is obtained at the oxidation potential of the metal, and the metal is said to be stripped from the mercury amalgam. As the potential sweep continues, the current falls back to the minimum until the oxidation potential for another element in the amalgam is approached and reached, when maximum current flows again. The magnitude of the peak current is proportional to the concentration of the metal in the original solution.

The use of x-ray fluorescence spectroscopy involves the irradiation of the sample with an unfiltered beam of high – intensity primary x-rays, which causes the elements present in the sample to emit their characteristic fluorescence lines. The sample should be preferably in a liquid form. The intensity of the emitted x-ray is influenced by the absorption and enhancement effects from elemental interactions and physical effects resulting from variations in particle size and surface [52]. Due to the above, a separation of the matrix elements is a necessary step in x-ray fluorescence analysis of environmental samples. Thick samples completely absorb the exciting radiation but fusing with sodium borate or mixing with silica gel are recommended methods for obtaining a uniform matrix [52]. However, by using a thin layer of samples deposited on a thin support which does not interfere, elements can be analysed at the nanogram levels, and special excitation such as with a small angle or polarized x-ray, lowers the detection limits even more. Concentration steps which produce thin layers include precipitation, adsorption and electrodeposition [52].

A resin material, chelex-100, has been used to concentrate Cu, Ni and Zn in seawater and the metals were determined directly on the resin using x-ray fluorescence spectroscopy [48].

Neutron activation analysis (NAA) on the other hand involves the irradiation of the sample with neutrons to produce radioactive or excited states of the elements in the sample. The advantage of NAA in maltielement trace determination is the high accuracy and precision when an instrumental analysis without separation is needed [52]. However, interferences in the α-spectra may arise from some of the matrix elements that are activated very highly. Such matrix elements have to be separated from the sample before determination of the trace metals. For example, sodium, potassium and phosphorus in large amount interfere in the α-spectra when trace heavy metals are determined using NAA.

2.5 Brief Chemistry of Heavy Metals Under Study

2.5.1 Chromium {Cr}

Chromium is a bluish – white, hard, shiny metal which is very resistant to chemical attack and not tarnished in air. Although it is unaffected by dry air at ordinary temperatures, some oxidation occurs on strong heating. It is the 24th element of the periodic table with electronic configuration – [Ar] $3d^5 4S^1$. Its commercially important ore is chromite [FeO. Cr_2O_3]. The principal valencies exerted by chromium are 3, 6 and 2 in decreasing order of stability, these being also the numbers describing the oxidation states. Chromium (III) is an essential trace element in mammalian metabolism and together with insulin, is responsible for the clearance of glucose from the blood stream. It forms stable salts with all the common anions and complexes with virtually any species capable of donating an electron pair. These complexes may be anionic, cationic, or neutral and with hardly any exceptions are hexacoordinate and octahedral e.g. $[CrX_6]^{3-}$ [X = halide, CN^-, SCN^-, N^-_3]. Hydrolyzed, polynuclear Cr (III) complexes are of considerable commercial importance in the dyeing (as mordants to dye) and tanning industries. Chromium is used in metal alloys and pigments for paints, inks, cement, paper, ceramics, rubber and other materials used as corrosion inhibitors and fungicides. Chromium can cause skin irritation and ulceration in low level exposure, damage to the kidney, liver, circulatory and nerve tissues on long-term exposure.

Potassium chromate, potassium tetraoxochromate (VI), may be estimated by conversion to dichromate on acidification and titration against ammonium iron (II) sulphate. It is used in analysis as an indicator for determination of chloride or bromide by silver nitrate in neutral solution, brick-red silver chromate not being precipitated by silver nitrate until all halide has been precipitated:

$$2Ag^+ + CrO_4^{2-} \rightleftharpoons Ag_2CrO_4\,(s).$$

This precipitation may be used as a qualitative test for chromate ions in neutral solution. Lead (II) acetate solution gives a precipitate of yellow lead (II) chromate with solutions of chromates. But hydrogen peroxide affords the most sensitive test; an intense blue colouration appears when hydrogen peroxide is added to an acidified chromate (or dichromate) solution. This is probably due to peroxide, possibly CrO_5, which may be extracted by ether as a blue immiscible layer.

2.5.2 Copper (Cu)

Copper is widely distributed in nature as a metal in sulphides, arsenides, chlorides and carbonates [53]. Age old methods like roasting and smelting or in some cases microbial assisted leaching are used for its extraction. Like many other metals, it is tough, soft and ductile. It is a reddish metal second only to silver in its high thermal and electrical conductivities. Here then underlies the importance of copper and its wide application in every day life [53]. It is also used in making brass and gold alloys.

Some physical constants [53] of copper are presented below:

Atomic Mass – 63.546

Electronic configuration – [Ar] $3d^{10}\,4S^1$

Melting point (K) – 1357.6

Boiling point (K) – 2836

Density at 300k (g/cm^3) – 8.96

Covalent radius (Å) - 1.17

Atomic radius (Å) - 1.57

Atomic volume (cm^3/mol) - 7:1

First ionization potential (V) – 7.726

Electrical conductivity ($10^6\,\Omega^{-1}\,cm^{-1}$) – 0.596

Thermal conductivity ($W\,cm^{-1}\,k^{-1}$) – 4.01

Crystal structure – cubic, face centered.

It is absorbed by plants as cupric ion (Cu^{2+}) and is a metal activator of several enzymes in plants, which include tyrosinase, luicase, ascorbic acid and oxidase. Deficiency of copper in plants cause unattractive appearance and smaller size of citrus fruits, discolouration of carrots, chlorisis and wilting in lettuce [54]. At high concentrations of 1 – 5 ppm, copper imparts an unpleasant taste to water and affect certain individuals suffering from a hereditary copper metabolism disorder called Wilson's disease [47].

Aqueous Chemistry of Copper

Copper exists in solution in two stable oxidation states of +1 and +2. However, the +2 oxidation state is the more stable. This explains the reason why mostly Cu (II) compounds exist.

Copper (I)

The copper (I) compounds are diamagnetic due to d^{10} configuration and except where colour results from the anion or charge transfer bands, they are colourless. In aqueous solution only low equilibrium concentration of Cu^+ ($< 10^{-2}$ M) can exist

$$2Cu^{I} \rightleftharpoons Cu + Cu^{II}$$

and the only Cu (I) compounds that are stable to water are the highly soluble ones such as CuCl or CuCN. This instability towards water is due partly to the greater lattice and solvation energies and higher formation constants for complexes of Cu (II) ion, so the ionic copper (I) derivatives are unstable.

The equilibrium above can be displaced easily in either direction and this makes it possible for CN^-, I^- and Me_2S to react with Cu (II) to give Cu (I) compounds. The residence time of Cu (I) in water depends strongly on the nature of the aqueous media.

Copper (I) forms complexes with simple ligands like halide ion, amine etc. The coordination in these complexes being absolutely tetrahedral.

Copper (II)

Most copper (I) compounds are readily oxidized to the copper (II) state because it is the most stable hence the most important oxidation state of copper. This also makes the aqueous chemistry of Cu (II) well defined. Various anionic salts which are hydrophylic including various complexes of Cu (II) exist.

Most Cu (II) salts dissolve in water to form copper – aqua complexes of the form, $[Cu(H_2O)_6]^{2+}$. If ligands are added to such an aqueous solution of Cu (II) competition for possession of the metal ion ensues between the water molecules and the ligand molecules and depending on how nucleophilic such a ligand is, complexes will be formed by successive displacement of the water molecules. This displacement reaction cannot be carried very far because after the addition of the fourth ligand molecule it becomes difficult to add more molecules because of Jahn – Teller effect which causes some distortion for cubic structured Cu (II) compounds. Based on the above reasons, the formation constants, K_5 and K_6, will have very small values[55].

2.5.3 Iron (Fe)

Iron is the second most abundant metal after aluminium and the fourth most abundant element in the earth's crust. The earth's core is believed to consist mainly of iron and nickel and the occurrence of iron meteorites suggests that it is abundant throughout the solar system. It is the 26th element of the periodic table with electronic configuration – [Ar] $3d^6\ 4S^2$. The major iron ores are haematite (Fe_2O_3), magnetite (Fe_3O_4), limonite [$FeO(OH)$] and siderite ($FeCO_3$).

Because of its high abundance, iron is often found as an impurity in other materials. For example corundum (γ-Al_2O_3) of gem quality is known as sapphire, and its colours are caused by small amounts of iron.

Iron is required in relatively large amounts due to the vital biochemical role it plays in the maintenance of life. It functions specifically in the activation of several enzymes such as fumaric hydrogenase, cetelase oxidase and cytochromes. It is also necessary for the synthesis of chlorophyll. In humans and animals, it is essential for the formation of haemoglobin in blood. The concentration of iron in water above 0.3 ppm can result in a reddish brown tinge and a bitter or astringent taste to water and thus can substantially affect the taste of beverages and stain cloths during washing [56].

Iron is a white, lustrous metal (m.pt 1528°C). It is not particularly hard, and it is quite reactive. In moist air it is rather rapidly oxidized to give a hydrous oxide that affords no protection because it flakes off, exposing fresh metal surfaces. Finely divided iron is pyrophoric. Iron combines vigorously with chlorine on mild heating and also with a variety of other non-metals including the other halogens, S, P, B, C and Si. The carbide and silicide phases play a major role in the technical metallurgy of iron.

Chemically pure iron can be prepared by the reduction of pure iron oxide (which is obtained by thermal decomposition of Fe (II) oxalate, carbonate or nitrate) with hydrogen by electrodeposition from aqueous solutions of iron salts, or by thermal decomposition of $Fe(CO)_5$.

The metal dissolves readily in dilute mineral acids, in the absence of air and with non-oxidizing acids to give Fe^{II}. With air present or when warm dilute HNO_3 is used, some of the iron goes to Fe^{III}. Very strongly oxidizing media such as concentrated HNO_3 or acids containing $Cr_2O_7^{2-}$ passivate iron. Air – free water and dilute air – free hydroxides have little effect on the metal, but hot conc. NaOH attacks it. In the presence of air and water iron rusts to give a hydrated Fe^{III}oxide.

Aqueous and Coordination Chemistry of Iron (II), d^6

Aqueous solutions of iron (II) in the absence of complexing anions contain the pale blue-green ion $(Fe CH_20)]^{2+}$, which is oxidized in acid solution'.

$2Fe^{2+} + ½ 0_2 + H^+ \rightarrow 2Fe^{3+} + H_20 \quad \varepsilon_0 = 0.46v$

Oxidation is easier in basic solution but neutral and acid solutions of Fe^{2+} oxidize less rapidly with increasing acidity (even though the potential of the oxidation reaction becomes more positive). This is because Fe^{3+} is actually present in the form of hydroxo complexes, except in extremely acid solutions, and there may also be kinetic reasons.

Complexes of Fe^{II}

Most Fe^{II} complexes are octahedral. There are few tetrahedral complexes but these including $(FeCl_4)^{2-}$ ion in salts with large cations, $(Fe(OPPh_2)_4)^{2+}$, and a few others. Pentacoordinate species, usually tribromophenol, include those of tripod ligands such as $(Fe(np)_3x)^+$. Dodecahedral complex include $(Fe(napthyridine)_4)^{2+}$ and crown ether complex.

The most important ligands, however, are porphyrins as many enzyme systems are iron porphyrins.

Aqueous and coordination chemistry of iron (111), d^5

Iron (111) occurs with most anions, except those with reducing capacity like the porphyrins[57]. Porphyrins which are complexes of conjugated macrocycles are of prime importance in living systems and for similar reasons other N_4 macrocyclic complexes have been intensively studied.

The square Fe^{II} porphyrins initially add oxygen reversibly but, unlike haeme and ligand potential systems, undergo autoxidation even at low temperatures to give μ-peroxo species, $(porphFe^{III})_2\mu\text{-}(O_2)$, which can react with a base to give (base)(porph) $Fe^{IV} = 0$.

The Fe^{IV} species are unstable decomposing to $(porph\ Fe^{III})_2\ \mu - 0$. The oxygenated species can oxidize organic substrates[53].

There are also other porphyrin compounds such as (porph)Feph, bridged species $(porphFe)_2x$; x = 0, N or C and carbenes, (porph) $Fe = CCl_2$, that are of the type implicated in CCl_4 and DDT toxicity. There are also other Fe^{IV} species such as (porph) Fe (x) ph, (porph) Fe = NR, and so on. Reduced Fe $(Oep)^{n-}$ species with Fe^0 and Fe^I are also known[57].

2.5.4 Zinc (Zn)

Pure zinc is a bluish-white metal of moderate strength which has excellent resistance to atmospheric corrosion. It is widely used as a surface coating on iron and steel to prevent rusting. Over the range of temperature 110°C – 150°C zinc is both malleable and ductile. In damp air, zinc is quickly covered by a coherent layer of basic carbonate. At red heat, zinc burns vigorously in air to form the fibrous zinc oxide, ZnO, once called philosopher's wool. Its combustion in the oxy-coal gas flame is spectacular.

Zinc is the 30th element of the periodic table with electronic configuration – (Ar) $3d^{10}4S^2$ and it is not a transition metal. The penultimate shell is complete both in the metal and in the ion, Zn^{2+}. Zinc is bivalent only and its ions are colourless. In compounds, zinc is of course, in the oxidation state +2.

It is an essential trace element for plants, animals and humans as it is associated with many enzymes and with certain other proteins. Zinc is relatively more abundant in the earth's crust (0.02%) than some other metals (e.g.Cu); however, there are not many minerals that contain Zn. There is only one common sulphide-Sphalerite (ZnS), but it forms minerals which are worked in many parts of the world. Anthropogenic sources of Zn in the environment include printing processes, construction materials, metals (iron, steel and brass coated with zinc), fertilizers, batteries, sewage-sludge, animal waste in the form of manure, Zn-containing pesticides (e.g. Zineb, Mancozeb and Ziram), atmospheric deposition and coal combustion. Zinc concentrations in soils typically range from 1 to 2000 $mgkg^{-1}$, but at some sites, levels as high as 10000 $mgKg^{-1}$ have been reported [47].

Although levels of Zn in soils are higher than those of copper, molybdenum and other micronutrients, plant requirements are also greater. It is required in plants for the activity of various enzymes (carbohydrate and protein metabolism) and for the synthesis of indole acetic acid (IAA)[54]. High levels of Zn are normally toxic to plants. Concentrations of Zn in natural waters are generally low, however, industrial effluents and drainage from mining areas can act as sources of pollution.

The major health concern in the general population is marginal or deficient Zn intake rather than its toxicity. Zn is generally considered as being of low toxicity due to the wide margin between usual environmental concentrations and toxic levels. However, high levels of Zn are undesirable as it may lead to Cu deficiency by inhibiting Cu absorption. In humans and animals, zinc deficiency causes dwarfism, reduces rate of blood clotting and wound healing, skin abnormalities, loss of appetite, severe growth depression and sexual immaturity[58]. In excess of 1.5ppm, it may impart astringent taste to water, and excessive ingestion of it causes carcinogenesis in humans[58].

Qualitative tests for zinc salts

Addition of caustic alkali to a solution containing Zn ions precipitate the white gelatinous hydroxide, which dissolves in excess alkali to form the zincate. Aqueous ammonia also precipitates the hydroxide, which dissolves in excess ammonia to give ammines. In the presence of ammonium chloride, suppression of the hydroxide ion concentration in aqueous ammonia prevents precipitation.

From neutral or alkaline solutions, hydrogen sulphide precipitates white (often discoloured) zinc sulphide, which is insoluble in weakly ionized ethanoic acid but readily soluble in mineral acids.

When zinc compounds, are mixed with fusion mixture, made from equal amounts of sodium carbonate and potassium carbonate, and heated on charcoal block, a yellow incrustation of the oxide, which becomes white as it cools, is formed. Addition of a little cobalt nitrate solution and further heating forms a green mass, Rinmann's Green, a solid solution of cobalt oxide in Zinc oxide.

Complexes of Zn^{II}

Of the complexes formed, only the ammines and cyanides are most important. Complex cyanides K $[Zn(CN)_3]$ and $K_2[Zn(CN)_4]$ are formed containing the anions, $Zn(CN)^-_3$ and $Zn(CN)^{2-}_4$, the tricyanozincate (II) and tetracyanozincate (II) ions respectively. The ammines may contain up to six molecules of ammonia for each zinc cation, $Zn(NH_3)^{2+}_6$. Passage of gaseous ammonia into a hot saturated solution of zinc chloride in water until the precipitate formed initially dissolves gives the tetraammine, which crystallizes on cooling as $[Zn(NH_3)_4]Cl_2$. On evaporation and cooling, the diammine is formed; $[Zn(NH_3)_2]Cl_2$. Passing dry ammonia over zinc chloride at-15°C yields voluminous mass of the hexaammine, $[Zn(NH_3)_6]Cl_2$.

2.5.5 Lead (Pb)

The proportion of lead by weight in the earth's crust is estimated as 0.002%. Galena, lead (II) sulphide, PbS, is the chief ore and is found associated with many other sulphides. Principal sources occur in Australia at Broken Hill (NSW) and Canada which are the two largest exporters, Mexico and the USA. Cerrusite, lead (II) carbonate, $PbCO_3$ and anglesite, lead (II) sulphate, $PbSO_4$, are other ores.

Lead, having a natural abundance of 13ppm[59] is by far the most abundant of the heavy metals, being approached only by Thallium (8.1ppm). This abundance is due to the fact that three out of the four naturally occurring isotopes of lead (206,207,208) arise primarily as the stable end products of the natural radioactive series. Only ^{204}Pb (1.4%) is non-radiogenic in origin.

The electronic configuration of lead with atomic number 82 is $[Xe]\ 4f^{14}\ 5d^{10}\ 6s^2\ 6p^2$ and is predominantly divalent; the tetravalent state tends to be strongly oxidizing in its compounds. In the divalent state, the two 6s electrons are inert due to inert pair effect, taking no part in chemical bonding. Lead (II) ion has ionic radius of 1.33Å and co-ordination number six. It exists in only one form which is metallic unlike tin which has three allotropes. Lead is now recognized as a heavy metal poison[60] (while Ge and Sn in the same group are non-toxic), it acts by complexing with oxo groups in enzymes and affects virtually all steps in the process of haeme synthesis and porphyrin metabolism. Pb (II) also inhibits SH enzymes [though less strongly than Cd (II)] especially by interaction with cystein residues in proteins. Typical symptoms of lead poisoning are cholic, anaemia, headaches, convulsions, chronic nephrites of the kidneys, brain damage, and central nervous system disorders[60].

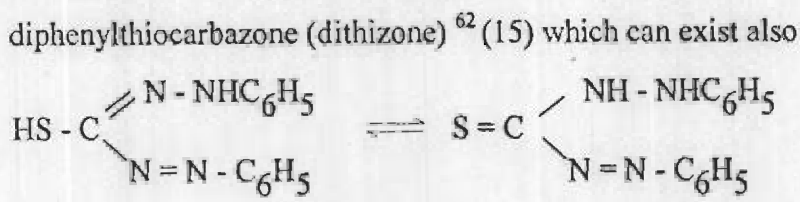
Uses of Lead

Lead is an extremely dense, grey metal (11.3gcm^{-3}) of low tensile strength, easily worked, being soft, malleable and with a low melting-point (327^{0}C). It is only superficially oxidized at ordinary temperature in air to hydrated oxide (hydroxide) and carbonate. Lead is used for sheathing electrical cables, as sheets for roofing and pipes for plumbing (Latin, plumbum=lead). Lead compounds as described above are poisonous. Some old homes may have lead water pipes, which can contaminate drinking water. In hard water districts, lead water pipes soon become coated with lead (II) carbonate and sulphate, preventing uptake of lead compounds. In areas served by soft, acidic, moorland water, silicates are added to water to give an insoluble coating of lead (II) silicate. Traces of lead in drinking water have a deleteriorus effect on health, retarding mental development. For this reason the use of tetraethyl lead, $Pb(C_2H_5)_4$, as an anti-knock agent in petrol is being phased out in countries of the European Union and elsewhere, particularly in the USA. Lead paints must be avoided where a child might chew the surface, as with cots, toys etc. Lead is highly resistant to sulphuric acid and is used in the Lead Chamber Process and as acid-resisting surfaces in the laboratories. Lead is used to absorb harmful radiation from nuclear reactors and X-ray sources. It was found that lead could inhibit nitrogen mineralization in the soil [61]. Alloying improves the tensile strength of lead and many solders and fusible alloys contain lead.

Complexes of Lead

Lead forms complexes with many organic reagents and has been used in its analysis.

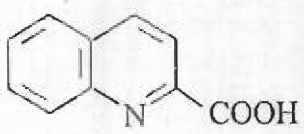
One of the most important reagents that has been in use for lead determination is diphenylthiocarbazone (dithizone) [62] (15) which can exist also as (16)



$$HS-C\begin{matrix}\nearrow\!\!\!\!= N-NHC_6H_5 \\ \searrow N=N-C_6H_5\end{matrix} \rightleftharpoons S=C\begin{matrix}\nearrow NH-NHC_6H_5 \\ \searrow N=N-C_6H_5\end{matrix}$$

(15) (16)

Other reagents include Quinaldic acid or its sodium salt (17) and (18), 8-hydroxyquinaldine (19) and 8-hydroxyquinoline (20)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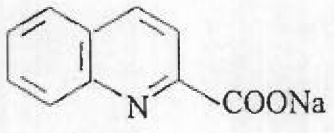
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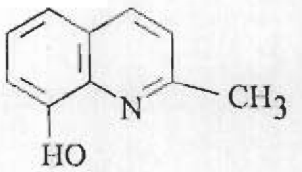
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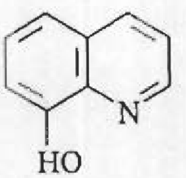
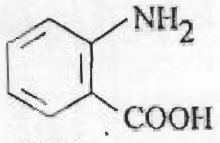

(20)

Cadmium (Cd)

The chief mineral is Greenockite, Cadmium Sulphide, CdS. Up to 0.4% cadmium may be found associated with znic and lead ores. Because of its volatility, cadmium accumulates in flues, electrostatic precipitators and other purification apparatus in lead, copper and zinc works. There is some alarm about ground water pollution by cadmium in certain nearby areas.

Cadmium has atomic number 48 with electronic configuration-[Kr] $4d^{10}$ $5S^2$ and density of $8.65 g/cm^3$. Cadmium (II) favours 4-coordinate tetrahedral complexes and to some extent 6-coordinate octahedral complexes. This is because stereochemistry of the compounds depend on the size and polarizing power of metal (II) cation (M^{2+}) and sterric requirement of the ligands since the d^{10} configuration affords no crystal field stabilization. Cd (II) ions do not form π complexes with CO, NO, or alkenes, because of the stability of its d^{10} configuration and its consequent inability to provide electrons for "back bonding". In solution, the majority of the complexes of Cd (like Zn) have a coordination number of 4 considered to be $[Cd(H_2O)_4]^{2+}$ which is poisonous to most living matter. Other complexes formed by Cd include, $[Cd(CN)_4]^{2-}$ $[CdCl_4]^{2-}$, $[Cd(NH_3)_4]^{2+}$ and they are tetrahedral in shape[63].

Cadmium can be determined as quinaldate using quinaldic acid or its sodium salt at a pH 5-7. Anthranilic acid (21) forms $Cd(C_7H_6O_2N)_2$ with cadmium



(21)

Diphenylthiocarbazone (dithizone) (15) can be made specific for cadmium, using a strongly basic solution. 8-Hydroxyquinaldine (19), 8-hydroxyquinoline (20) can also quantitatively precipitate cadmium when the pH is adjusted. Chelating agents are weak acids; the number of elements precipitated can usually be regulated by adjustment of the pH [13]. The reaction can be generalized as:

$M^{n+} + nHX = MXn + nH^{+}$

The weaker the metal chelate formed, the higher the pH needed to achieve precipitation.

Cadmium has no known biological role and is among the most toxic of all elements [64]. It accumulates in humans mainly in the kidney and liver leading to dysfunction of the kidneys, cardiovascular diseases, hypertension and sterility among males. It can inhibit the action of zinc enzyme by displacing the zinc. It can also replace calcium in bones, leading to painful disorder and even death.

Uses of Cadmium

Cadmium is a silvery-white, soft, ductile and malleable metal. Electroplating is the chief outlet of cadmium. Small steel articles are protected from rusting in a way similar to the protection afforded by zinc. Cadmium-plating is applied to radio and telephone components made of copper and brass. Cadmium may form the negative plate of alkaline electrical storage cells with an electrolyte of caustic potash. In nuclear engineering, cadmium rods have been used in the regulators of atomic piles for the conversion of Uranium into plutonium.

Cadmium is a constituent of certain bearing alloys, solders and fusible alloys. It is used to strengthen copper for long spans of the high-voltage overhead electric cables of the National Grid.

2.6 Preparation of Water Sample for Trace Metal Determination by Atomic Absorption Spectrophotometry

High salt concentrations in saline waters preclude the direct use of atomic absorption spectrophotometry in determining trace metals in water, using flame atomization (flame – AAS). This is due to scattering effect on the flame and the clogging of the burner by the salt particles. In addition, these metals occur in natural waters in very trace amounts. Flameless-AAS using electrothermal atomization (ET-AAS) is also rendered unreliable by high salt content even though the technique is sufficiently sensitive for direct application [50].

Usually, the trace metals are concentrated from a large volume of water and the transition metals separated from the alkali and alkaline earth metals. In practice, pre-concentration and separation are conveniently combined in such techniques as liquid-liquid extraction (chelation/extraction), co-precipitation, use of chelating or ion exchange resins and adsorption on adsorbents such as carbon [50].

Chelation followed by extraction have had the widest applications in trace metal analysis of solutions. Usually, an organic chelating agent such as ammonium pyrolidene dithiocarbamate (APDC), 8-hydroxyquinoline (oxine), dithiozone, cupferron, etc. is used to form chelates with the trace metals which are mostly transition metals. Such chelates are soluble in organic solvents like methyl isobutyl ketone (MIBK), Chloroform, amylalcohol, ethyl acetate [47] etc. Chloroform is however no more in use because it has been found to be carcinogenic.

2.7 Liquid – Liquid Extraction

Since the discovery that ether could extract uranium (VI) from nitric acid, the study of solvent extraction techniques has become wide and varied both in scope and application. To lay credence to the versatility of solvent extraction as a veritable analytical tool, skey [65] posited an elegant method for the separation of cobalt from nickel, gold from platinium and iron from many other metals by coveting the phenomena of different solubilities of their thiocyanate solutions in ether.

Despite the establishment of new and easier solvent extraction systems and procedures, new organic reagents have also been prepared to make solvent extraction a viable field for analytical and inorganic chemists. The introduction of a versatile organic reagent, "dithizone" followed by the application of cupferron, dimethylglyoxime about five years later and 8-hydroxyquinoline [66] in the 1940s opened a new era in liquid-liquid extraction studies which was neglected from 1900 till then.

In comparison to the phenomenon of liquid-liquid extraction of ion-pair associated complexes, which is selective and for which the physico-chemical interpretations are so intrinsically complicated, the interaction of cations with suitable organic chelating agents to give formally neutral and less hydrophilic entities provides an immediate clue to their role in promoting the solvent extraction of inorganic species [67].

A thermodynamic interpretation of the phenomena of liquid-liquid distribution equilibria was introduced by Kolthoff and Sandell[68]. Their work established unambiguously very easy means of relating the percentage of metal extracted to pH of aqueous phase, concentration of organic reagent or the concentration of auxiliary masking agents. These equations, from practical usage, have been established to apply to all solvent extraction systems.

Studies on many methods of analysis whereby the transport of materials from one phase to another – the most fundamental procedure for the separation of a chemical species from the medium – has spanned many decades. From these studies, methods like distillation and condensation, dissolution and crystallization or precipitation and sublimation have been discovered, utilized and perfected. When compared to solvent extraction, these methods are found wanting. The versatility and scope of solvent extraction as a sort of interphase transport process is founded on its ease of application, speed and simplicity [67]. In solvent extraction, separation is cleaner when compared to the precipitation method. Solvent extraction procedures are ideally suited for the isolation of trace quantities of species in a sharp contrast to the precipitation process [4]. In addition, the greater variety of extractants makes it possible for an easy selection of most suitable system for any given problem.

Employment of solvent extraction in tackling a chemical problem will require very common and non-complicated apparatus like a small extraction bottle, or a separation funnel. It also requires short time that can range from a few minutes to some hours for separation of chemical species to be achieved. Therefore, it is one of the most suitable methods of separating both micro and macro quantities from large amounts of other substances since such undesirable phenomena as co-precipitation encountered in methods like precipitation is non-existent.

Judicious utilization of reagents and control of pH makes the method highly selective, and the isolation of the metal in question can be made as complete as desired by several repetition of the extraction procedure. Also, as most chelates are coloured, spectrophotometric methods are employed in detection and quantification of trace amounts of metals.

Solvent extraction can now be applied effectively in chemical technology, in purifying chemical reagents, also in separating several radioisotopes and processing nuclear fuels [67].

2.7.1 Distribution Law

It has been established that the basis of solvent extraction is a two-phase distribution of molecular species. Therefore, the classical phase rule of Gibbs applies, viz:

$$P + F = C + 2 \quad \text{-------------------------------------} \quad (1)$$

Where P is the number of phases, F the number of degrees of freedom and C the number of components. Equation (1) shows that a system consisting of two immiscible solvents and one solute distributed between them has one degree of freedom at constant temperature and pressure. Therefore, if the concentration of the solute in one phase is constant, the concentration of the solute in the other phase is also fixed [67].

Theoretically, this concept can always be applied to any solvent extraction system. It was on this basis that Nernst formulated the "distribution law", which is a general principle for relating the concentration of the solute in each phase. This law has become the most fundamental rule in solvent extraction chemistry.

For a system in equilibrium where a solute distributes itself between two immiscible phases, Nernst distribution law states that at a particular temperature and with the solute existing in the same molecular state in both phases, the ratio of the activities of the solute in both phases is invariant (ie) it will always be constant and independent of the total concentration. This constant is called the distribution or partition coefficient, K_D, of the solute in the solvent. Hence, if the activity of the solute in the organic phase is denoted as Aorg and its activity in the aqueous phase denoted as Aaq where "org" and "aq" represent organic and aqueous phases respectively, the partition coefficient can be represented mathematically as

$$K_D = \frac{A_{org}}{A_{aq}} \quad \text{-------- -- --} (2)$$

The activity A is represented as A = Yc where Y is the activity coefficient and C is concentration. For very dilute solutions, Y tends to unity, therefore:

$$K_D = \frac{C_{org}}{C_{aq}} \quad \text{------------------------} \quad (3)$$

This equation shows that the partition coefficient does not depend on the total solute concentration or on actual phase volumes.

The law however, can only have real theoretical significance but not useful practically as it cannot account for such phenomenon as dissociation or association of the solute in solution whereby it exists in other molecular states as is normally encountered in solvent extraction practice. A practical term in use is the distribution ratio, D:

2.7.2 Distribution Ratio, D

Defined, as the ratio of concentrations of solute in all its forms in the two phases, distribution ratio takes into account the fact that a solute exists in a two phase system in several chemical forms M_1, M_2 ----- M_n. However, only the total concentration of the solute in each phase can be experimentally measured, hence the distribution ratio for these systems can be denoted as

$$D = \frac{[M]\, org, total}{[M]\, aq, total} = \frac{M_1\, org + M_2\, org + --- M_m\, org}{M_1\, aq + M_2\, aq + --- M_m\, aq} --- \quad (4)$$

In some cases, the distribution ratio differs with different experimental methods. This arises from the fact that one method may detect and measure an element for example, in two chemical states, while another method can do so in only one chemical state. The distribution ratios calculated from the data obtained from these two methods would be different from each other.

2.7.3 Percentage Extraction, E

For thermodynamic study of distribution equilibria, distribution ratio is always advantageous but extraction can be conveniently expressed as the percentage extraction, E. This gives the percentage of the metal extracted into the organic phase. This quantity is defined by

$$E = \frac{Vorg\, Morg}{Vorg\, Morg + Vaq\, Maq} X\, 100 ------------------ (5)$$

and the relationship between the distribution ratio and the percentage extraction is given by the following equation.

$$E = \frac{D}{D + \left(\frac{Vaq}{Vorg}\right)} X\,100 \text{ ------------------------- } (6)$$

where V stands for phase volume.

To easily consider the distribution equilibrium of a material, it is preferable to express it as D, although E gives a clearer picture of the efficiency of that operation in analytical or preparative chemistry, especially when the volumes of the two phases are different. Of utmost importance in liquid-liquid extraction is the transference of as much metal species as possible from the aqueous phase to the organic phase so as to obtain both high D and E values. It is equally important to device a method for the recovery of the metal from the organic phase since it is of immense economic importance in the industry.

2.8 Classification of Inorganic Extraction Systems

Inorganic extraction systems have been identified to exist in three forms; as metal chelates, ion-pair or ion-association complexes, and additive complexes. These are all neutral metal species that can form during solvent extraction of metals.

Metal Chelates

When a metal M with charge n^+ is equilibrated with organic and aqueous phases, first, the metal ion is solvated in the aqueous phase by water molecules forming metal-aquo complexes. In the presence of an organic reagent HA, which will complex the metal ion, the aquo complex has to be destroyed for the complexation to take place.

For the organic reagent to be a chelating agent, it must behave like a weak acid. In this wise, the ability to ionize in the aqueous media forming a proton and conjugate base of the weak acid is necessary. It is in this form that a displacement reaction can take place whereby the water molecules attached to the metal are substituted by the conjugate base groups (chelate ligands) in a ratio that gives a neutral compound (metal chelate).

Organic reagents having one anionic group (e.g. – OH, - SH, etc) and one uncharged basic group (e.g. = N - , = O, etc.) qualify as chelating ligands and can easily replace co-ordinated water molecules from metal ions and provide more than one point of co-ordination to the metal ion forming chelate compounds which are essentially neutral and covalently bonded. Chelate compounds, in which the metal has become part of the organic structure and is more or less buried inside the molecule, are mostly hydrophobic (having little solubility in water) but organophilic (dissolve readily in organic solvents). This behaviour makes it possible for metal chelates to be extracted into the organic phase.

2.8.2 Ion – Pair Complexes

Ion – pair or ion – associated complex is formed when an anionic complex of a metal ion interacts with cationic species formed by the dissociation of ligand in the aqueous phase which must be acidic. For such a complex to form therefore, more than one step must be involved.

First, the aquo molecules attached to the metal ion are replaced by the predominant anions in the aqueous phase to give an anionic complex. Secondly, on equilibration the organic reagent transfers to the acidic aqueous phase where it is protonated and it becomes cationic. Lastly, the anionic metal complex associates with the cationic ligand to form an ion-pair complex. Since the interaction between the cation and anion is essentially electrostatic and there is no strong solvation in the organic phase, the complex is easily extracted into the organic phase. High diaelectric solvents like nitrobenzene and dichloromethane are required for this complex to be formed.

2.8.3 Additive Complexes

These are complexes originally formed either in ion-associated or in chelate form but in addition may have organic solvent molecules, reagent molecules, or hydroxyl molecules coordinated to the metal as ligands.

(i) Additive Complexes with Organic Solvents

Additive complexes are mostly formed when hydrophobic reagents such as trion-butylphosphate or trion-butylphosphine oxide are employed as organic solvents, and, or the maximum co-ordination number of the metal and the geometry of the ligands are favourable. In the first case, the organic reagent (solvent) can replace water molecules attached to the metal chelate to form a less hydrophilic additive complex which is readily extracted by non-polar solvents [69]. This behaviour recognized as a sort of synergistic enhancement of the distribution ratio operates generally where the following conditions are fulfilled [70].

(a) Where the organic reagent readily displaces residual coordinated water molecules from the neutral chelate rendering it less hydrophilic.

(b) Where the organic reagent is less nucleophilic and less hydrophilic than the chelating ligand, and

(c) Where the maximum coordination number of the metal and the geometry of the ligands is favourable.

The existence of such a complex is readily established by measuring the distribution ratio D_1 and D_2 of the metal using two different organic solvents at the same reagent concentration. If additive complex is formed with organic solvent, the ratio D_1/D_2 will vary with the pH otherwise it will remain constant [71]. This means that log D_1 and log D_2 when plotted as a function of pH will run parallel for no formation of additive complex.

(ii) Additive Complexes with the Organic Reagent

These have the general formulae of $MA_n (HA)_r$ and $MA_n (OH)_p (HA)_r$ where M is the metal ion, n its charge, HA the organic reagent whereas p and r are the number of hydroxyl and ligand molecules coordinated to the complex. The media must be highly hydrolytic for the latter complex to be formed while the former one is not affected by the pH of the media. Formation of both complexes will be possible where the metal coordination number and the geometry of the ligands are favourable and the reagent concentration high enough.

(iii) Additive Complexes with Hydroxyl Groups

These complexes have the general formula $MA_n (OH)_p$ and will require highly alkaline media to effect hydrolysis of the metal ion. Extraction of such complexes will require organic solvents with high dielectric constant since they are not neutral complexes. Consequently, they will give low partition coefficient in organic solvents with low dielectric constant.

Factors that Influence Stability and Extractibility of Metal Chelate Complexes

Kolthoff and Sandell [68] were the first to quantitatively investigate the process of solvent extraction. After their work, other workers like Morrison and Freisser [72] extended the frontiers of liquid-liquid extraction of metals by providing more detailed overview of the factors that come to bear on its workability.

(i) Influence of Acidity (pH)

Chelate formation as often encountered in solvent extraction arises from interaction between an organic reagent HA and metal ion M^+ and can be pictured as [73].

$$M^{n+} + nHA\,(org) \rightleftharpoons MA_n(org) + nH^+ \tag{7}$$

Where MA_n (org) is the metal chelate formed. From equation (7) we can deduce the equilibrium constant or extraction constant, Kex of the reaction as

$$K_{ex} = \frac{[MA_n]\,org\,[H^+]^n}{[M^{n+}]\;\;[HA]^n\,org} \tag{8}$$

From equation (4), the distribution ratio D is given by Morg/Maq, hence equation (8) changes to

$$K_{ex} = D\,X\,\frac{[H^+]^n}{[HA]^n} \tag{9}$$

For any given system at a particular temperature, K_{ex} is constant and depends mostly on the ionic strength of the aqueous media. If the concentration of the organic reagent is kept constant i.e. [HA] = constant (C) equation (9) becomes

$$K_{ex} = D\,X\,\frac{[H^+]^n}{C} \tag{10}$$

therefore, $$D = \frac{K_{ex}\;X\;C}{[H^+]^n} \tag{11}$$

which implies that $$D \propto \frac{1}{[H^+]^n} \tag{12}$$

Equation (12) depicts that the distribution ratio varies inversely as the exponential power of the hydrogen ion concentration.

Taking the log of equation (9) it will be obtained that

$$\text{In}\ K_{ex} = \text{In}\ D + \text{nln}\ [H^+] - \text{nln}\ [HA]\ \text{org} \text{ ------------------------------------ } (13)$$

This implies that

$$\text{In}\ D = \text{In}\ K_{ex} + 2.303\ n\ \text{pH} + n/n\ [HA]\ \text{org} \text{ -------------------------------- } (14)$$

$$D = K_{ex}\ [HA]^{n}\ \text{org}\ e^{2.303n\text{pH}} \text{ -- } (15)$$

It has already been established in equation (9) that when a metal ion M^{n+} reacts with an organic reagent HA to give an uncharged metal chelate MA_n, the extraction constant, K_{ex}, has a direct relationship with the distribution ratio, D, of the metal. From equation (15), the relationship between the distribution ratio and the acidity can be deduced. This equation shows that D increases exponentially with pH for the extraction of the metal chelate. A plot of D against pH gives a curve of which the slope depends on n and the position along the pH axis depends on K_{ex}.

(ii) Influence of Masking Agents

A masking (sequestering) agent is a compound or anion which when present in the aqueous phase can form a non-extractable complex with the metal ion. There are many examples of these but the most common ones are EDTA, citrate, cyanide, fluoride, thiocyanate etc. In the presence of the masking agent, the distribution ratio of the metal decreases because less quantity of the metal would be available to go into the organic phase than would have been possible in its absence.

This phenomenon of masking is very important in solvent extraction chemistry because by judicious utilization of masking agents a particular metal of interest can be separated from a mixture of other metals that form non-extractable ionic complexes and remain masked in the aqueous phase. The extracted metal either forms unstable complex with the masking agent or does not react at all with it hence its preference for formation of the extractable chelate with the organic reagent.

(iii) Influence of the Concentration of the Organic Reagent

Equation (15) establishes the fact that the extractability of a metal with a given organic reagent and organic solvent depends largely on the concentration of the organic reagent. It is clear from this equation that the distribution ratio increases with increase in concentration of the organic reagent, and the extraction curve is shifted to the acid side permitting extraction from more acidic solutions. Therefore, in systems where hydrolysis of the metal comes about readily the use of high reagent concentration becomes very necessary.

The solubility of the reagent in the organic solvent and the formation of non-extractable hydroxyl complexes puts a limit to the extent to which this phenomenon may be utilized. Besides, excess reagent may pose a great problem in the spectrophotometric determination of the metal chelates where the reagent has high extinction coefficient at the wavelength of measurement. On the other hand, excess reagent is utilized to ensure quantitative extraction.

(IV) Influence of Metal Oxidation State

A metal may readily form chelate complex with an organic reagent in one oxidation state, but not in another oxidation state. In such cases the metal must be converted to the suitable oxidation state before being extracted. Examples include Pd^{2+} forming complex with dithizone while Pd^{4+} does not or Fe^{2+} being easily complexed with O-phenanthroline whereas Fe^{3+} would not.

(V) Influence of.Organic Solvent

The organic solvent which serves as a diluent for the organic reagent must have sufficient miscibility with the organic reagent. It should also in addition have the following properties.

It should have very low viscosity, and also the specific gravity must differ so much from that of the aqueous phase in order not to form emulsions.

It should have low toxicity and inflammability.

It should be practically immiscible with the aqueous phase so as to make for high distribution ratio during extraction.

The metal chelate should be readily soluble in it.

The organic solvent should have such a property that will make for easy stripping of the metal chelate.

(VI) Influence of Salting-out Agents

Salting-out agents are ionic salts usually of common anion with the acid under investigation. They enhance the distribution ratio of the metal by the following mechanisms [66].

Hydration theories: salting-out action comes about by the effective removal of water molecules from their solvent role due to the hydration of the salt. In this way, free water molecules are depleted paving way for easy formation of the metal chelates.

Electrostatic theories: the water molecules are thought of as constant dielectric hence only the electrostatic interaction of the ions can change the ideality of the solution. This action increases the dielectric constant of the aqueous media thereby reducing the solubility of the metal chelate therein.

The salting-out action results from compression of the solvent medium especially when it interacts with a neutral solute.

The ionic activity of the extractable metal chelate is also decreased.

(VII) Influence of Solubility of Metal Chelate

The distribution coefficient of a metal chelate, MAn, approximates correctly to the ratio of its solubility in both phases. Thus, metal chelates formed by hydrophilic groups like oxalate, tartarates, citrates etc. which are readily soluble in the aqueous phase and practically insoluble in the organic phase will not be extracted into the organic phase. Metal chelates like acetylacetonates of Zinc, cobalt (II), nickel, manganese (II) etc. which are soluble in both phases can only give partial extraction. On the other hand, those chelates that are hydrophobic and readily soluble in the organic phase can be quantitatively extracted. Based on the solubility data of these chelates, it is possible to find the most suitable solvent for extraction.

CHAPTER THREE
EXPERIMENTAL

3.1 Description of Apparatus

Weighings: Mettler Balance, type P162N, was used for all the weighings.

pH Measurements: The pH values of buffer solutions were monitored using Universal pH indicator paper and digital pH meter type pHs – 25.

Separation of Phases: Phase separation was done using suitable separatory funnel.

Spectral Instruments/Measurements: Absorbances of solutions were measured with Unicam 939/959 – Solaar Atomic Absorption Sepctrophotometer.

UV – Visible Measurements: Electronic spectra were measured with Unico UV – 2102 PC spectrophotometer.

Other Apparatus: Rotary Evaporator, Vacuum Pump, Thermometer, a set of refluxing apparatus and KADIO $^{(R)}$ KD – 1069 stopwatch.

3.2 Methodology

The determination of trace metals in saline waters using flame atomic absorption spectrophotometry requires pre-enrichment and separation of the trace metals from the major cations whose salt particles in the flame cause scattering of the flame which lead to unreliable results. Commonly, ammonium pyrolidene dithiocarbamate/methyl isobutyl ketone (APDC/MIBK) chelation/extraction system is used for this purpose, but, since these reagents were not easily available, the extraction procedure involving cyanidin chloride/1-butanol system was studied and used in the current work. The procedure is similar to the oxine/chloroform method earlier used by Okoye[5] also, it is an advancement of the method used by Gentry and Sherrington[24] who extracted individual metals at different pH while in the current procedure, all the metals of interest were simultaneously extracted at pH7. The procedure showed high enough efficiency, reproducibility and speed.

The best technique for evaluating an analytical procedure is the analysis of one or more standard samples whose composition with respect to the analyte (s) of interest is reliably known. However, for the technique to be of value, it is essential that the standards closely resemble the samples with respect to the concentration range of analyte(s) and the overall composition of the samples (the matrix). If the samples are environmental ones, it usually becomes impossible to acquire standard samples which closely match the field samples, in which, of course, the form of the analyte(s) is unknown or variable and quite impossible to reproduce. Under such situations, the standard-addition technique is adequate[5].

In standard-addition technique, a series of solutions containing both the sample and varying concentrations of the substance to be determined are prepared by adding aliquots of a standard solution to the sample. The solutions are analysed and the response of the instrument is plotted against the concentration due to the added standard. This method is used to eliminate matrix effects, ie, interferences by other components which may be present in the samples.

3.3 Extraction of 3, 3^1, 4^1, 5, 7 – Pentahydroxyflavylum Chloride

About 400g of the flowers of *Hibiscus rosa-sinensis* (L) (Malvaceae) were collected from within University of Nigeria, Nsukka campus. The flowers were macerated in 2.0 dm^3 of (85:15 $^v/v$) methanol – 2M HCl mixture for 72 hours to extract the pigment. The extract after filtration was concentrated to about 500ml using the rotary evaporator, filtered again into a beaker and about 100ml of concentrated HCl added to it. This was heated in a round bottom flask under reflux for about three hours. The refluxed solution was transferred into a covered beaker and then cooled in a refrigerator until crystals settled out. The crystals were filtered out under suction, recrystallized several times from methanol, air dried and weighed. The yield was thereafter calculated using the expression:

$$\text{Percentage yield} = \frac{mass\ of\ dry\ crytals}{mass\ of\ flower} X \frac{100}{1}$$

The air dried compound was stored in a dark sample bottle to avoid auto – oxidation.

3.4 Solubility Test

To obtain a non-water miscible organic solvent which is also non-viscous, non-toxic and lowly inflammable that can be used for extraction with 3, 3^1, 4^1, 5, 7-Pentahydroxyflavylium chloride (cyanidin chloride), the solubility of cyanidin chloride crystals was tested on the following organic solvents: acetone, methanol, ethanol, hexane, toluene, carbon tetrachloride, 1-butanol, diethylether and ethylacetate.

3.5 Spectral Characterization of the Sample

80mg of cyanidin crystals were dissolved in 100ml of methanol containing 0.01% HCl. Subsequently the UV and visible spectra of the solution were determined using Unico UV – 2102 PC spectrophotometer .The process was repeated using 1-butanol as solvent for both the free ligand and the ligand complexed with solutions of lead,chromium and cadmium ions.

3.6 Qualitative Tests for Cyanidins

Freshly extracted solution of cyanidin from Hibiscus flower in 1.0% aqueous HCl was used for the following tests:

Cyanidin Reagent: A portion was shaken with an equal volume of a mixture of cyclohexanol and toluene (1:5 $^v/v$) and the colour observed.

Oxidation Test: A portion was shaken in air and half its volume of 10.0% sodium hydroxide was added. This was immediately followed by the addition of concentrated HCl and amylalcohol to determine whether the anthocyanidin can be recovered.

Colour Test: To a portion extracted with amylalcohol, sodium acetate was added and the colour was observed. This was followed by the addition of a drop of ferric chloride solution with shaking and the colour again observed.

3.7 Preparation of Metal Stock Solutions

The metal stock solutions were prepared fresh weekly (to avoid formation of hydrolytic species) with oven dried salts as follows:

Chromium:- A stock solution of chromium containing 10,000 mg/l was prepared by dissolving 2.830g of potassium dichromate, $K_2Cr_2O_7$, in minimum volume of concentrated nitric acid and diluting to 100ml with 1% $^v/v$ HCl.

Cadmium: 1.536g of cadmium carbonate, $CdCO_3$, was dissolved in 1.50ml of 1:1 HCl and made up to the 100ml mark with 1% $^v/v$ HCl to give a 10,000mg/l stock solution of cadmium.

Lead: Lead stock solution (10,000mg/l) was prepared by dissolving 1.600g of lead nitrate, $Pb(NO_3)_2$, in 1.5cm^3 of concentrated HNO_3 and made up to the 100ml mark with distilled water.

Copper (II): Stock solution of copper (II) containing 10,000 mg/l was prepared by dissolving 3.908g of copper sulphate pentahydrate, $CuSO_4.5H_2O$ in 10ml of distilled water and made up to 100ml mark with distilled water.

Zinc: 1.002g of zinc metal was dissolved in 20ml of 1:1 HCl, and made up to 100ml mark with 1% $^v/v$ HCl for the zinc stock solution containing 10,000mg/l.

Iron: 1.000g of iron metal was dissolved in 50ml of 1:1 HNO_3 and made up to 100ml with 1% $^v/v$ HCl for the iron stock solution containing 10,000mg/l.

3.8 Preparation of Standard Solutions of Salts and Acids [74]

(i) 0.2M KCl: 1.492g of oven dried KCl was weighed into a 100ml standard flask, dissolved in small quantity of distilled water and made up to mark.

(ii) 0.2 M HCl: 1.8ml of 36.0% HCl was made up to $100cm^3$ with distilled water.

(iii) 0.1 M HCl: 9.0ml of 36.0% HCl was measured into a standard flask and made up to $1dm^3$ mark with distilled water.

(iv) 0.1M NaOH: 10.0g of NaOH was dissolved in distilled water and made up to 250ml mark with distilled water.

(v) 0.1M KHphthalate: 2.05g of KHpthalate was dissolved and made up to 100ml mark with distilled water.

(vi) 0.1M KH_2PO_4: 1.742g of KH_2PO_4 was dissolved in distilled water and made up to 100ml mark.

(vii) 0.1M H_3BO_3: 1.551g of oven dried Boric acid was dissolved in a minimum volume of distilled water and made up to 250ml mark.

3.9 Preparation of Buffer Solutions [75]

Buffer solutions of pH 1 – 10 were prepared as described in Langes Handbook of Chemistry with the above standard solutions of salts and acids. The pH values of the buffer solutions were monitored using Universal Indicator papers and Digital pH meter of the type pH –25.

pH 1: 25 ml of 0.2M KCl was pipetted into a 100ml standard flask, 67ml of 0.2M HCl was added and made up to 100ml mark with distilled water.

pH 2: 25ml of 0.2M KCl was pipetted into a 100l standard flask, 6.50ml of 0.2M HCl was added and diluted to 100ml mark with distilled water.

pH 3: 50ml of 0.1M KHPhthalate was pipetted into a 100ml standard flask, 22.50ml of 0.1M HCl was added and diluted to 100ml mark with distilled water.

pH 4: 50ml of 0.1M KHPhthalate was pipetted into a 100ml standard flask, 2.80ml of 0.1M NaOH was added and diluted to 100ml mark with distilled water.

pH 5: 50ml of 0.1M KHPhthalat was pipetted into a 100ml standard flask, 22.60ml of 0.1M NaOH was added and made to 100ml mark with distilled water.

pH 6: 50ml of 0.1M KH_2PO_4 was pipetted into a 100ml standard flask, 5.60ml of 0.1M NaOH was added and diluted to 100ml mark with distilled water.

pH 7: 50ml of 0.1M KH_2PO_4 was pipetted into a 100ml standard flask, 29.10ml of 0.1M NaOH was added and diluted to 100ml mark with distilled water.

pH 8: 50ml of 0.1M KH_2PO_4 was pipetted into a 100ml standard flask, 46.10ml of 0.1M NaOH was added and made up to 100ml mark with distilled water.

pH 9: 50ml of a mixture 0.1M with respect to both KCl and H_3BO_3 was pipetted into a 100ml standard flask, 20.80ml of 0.1M NaOH was added and made up to mark with distilled water.

pH 10: 50ml of a mixture 0.1M with respect to both KCl and H_3BO_3 was pipetted into a 100ml standard flask, 43.70ml of 0.1M NaOH was added and diluted to 100ml mark with distilled water.

3.10 Equilibration Temperature

All extractions were carried out at room temperature 27±1°C. Solutions of both organic and aqueous phases were allowed to equilibrate at room temperature before mixing for extraction.

3.11 Equilibration Time

The time required for the metal complex transfer from the aqueous to the organic phase to reach equilibrium was investigated in buffer solution as follows:

10cm^3 of a mixed standard solution containing 100μg/cm^3 Cu ions was pipetted separately into nine different 100ml separating funnels , 25cm^3 of buffer solution pH 7 and 25cm^3 of cyanidin chloride [3, 3^1, 4^1, 5, 7-Pentahydroxyflavylium chloride] in butan 1 – ol were added to each funnel and the pH adjusted to 7 with either dilute ammonia solution or hydrochloric acid and finally made up to 100cm^3 with distilled water. The separating funnels with their contents were shaken vigorously for the following time intervals respectively: 30 secs, 60 secs, 90 secs, 120 secs, 150 secs, 180 secs, 210 secs, 240 secs and 270 secs which was monitored with KADIO ® KD – 1069 Stop Watch. The solutions were allowed to settle and the phases later separated. The amount of Cu left unextracted was determined spectrophotometrically using the Unicam 939/959 atomic absorption spectrophotometer. The equilibration time was determined as the time after which no further increase in the extraction is observed.

3.12 Extraction from Buffer Solutions

Serially diluted mixed standard solutions were prepared by pipetting the appropriate volume of each stock solution into different 100ml separating funnels labeled pH 1 – pH 10. 25cm^3 of the corresponding buffer solution was added followed by 25.0cm^3 of 2% 3, 3^1, 4^1, 5, 7-Pentahydroxyflavylium chloride [cyanidin chloride] solution in butan -1-ol. The solutions were adjusted to the appropriate pH using either dilute HCl or ammonia solution and finally made up to mark with the corresponding buffer solution such that each solution contained the metals in the following concentrations: Cd (2ppm), Zn (3ppm), Cr (3ppm), Cu (5ppm), Fe (5ppm) and Pb (20ppm) respectively.

Each of the mixtures was equilibrated for three minutes and allowed to settle in the separating funnel before the phases were separated. The amount of metals in both the organic and the aqueous phases were determined spectrophotometrically using the Unicam 939/959 atomic absorption spectrophotometer against the blank [2% cyanidin chloride in butanol] and the different concentrations of mixed standard solutions. The percentage recovery of each metal at a given pH was calculated as:

$$\frac{\textit{Concentration in the organic phase}}{\textit{Initial concentration}} X\,100\%.$$

3.13 Heavy Metal Recovery Experiment in pH Range 5.0 – 7.0

Five different serially diluted mixed standard solutions for a quintet [5 times] analyses were prepared such that the mixed standard solutions [MS] contained the metal ions in the following concentrations;

MS$_1$: Zn (1ppm), Cd (1ppm), Cr (1ppm), Cu (1ppm), Fe (1ppm) and Pb (2ppm).
MS$_2$: Zn (2ppm), Cd (2ppm), Cr (2ppm), Cu (2ppm), Fe (2ppm) and Pb (4ppm).
MS$_3$: Zn (3ppm), Cd (3ppm), Cr (3ppm), Cu (3ppm), Fe (3ppm) and Pb (6ppm).

MS_4: Zn (4ppm), Cd (4ppm), Cr (4ppm), Cu (4ppm), Fe (4ppm) and Pb (8ppm).

MS_5: Zn (5ppm), Cd (5ppm), Cr (5ppm), Cu (5ppm), Fe (5ppm) and Pb (10ppm).

The extractions were done as described above using 2% cyanidin chloride solution in butanol and the pH adjusted to 5.0, 5.5, 6.0, 6.5 and 7.0 respectively by the careful dropwise addition of either dilute HCl or dilute ammonia solution as the case may be. Equilibration was for three minutes and separation done after the phases had settled out.

The amount of metals in both the organic and aqueous layers were determined as before using the same model of Atomic Absorption Spectrophotometer. The percentage recoveries of each metal were calculated. The mean percentage recoveries for the five extractions with their standard errors and coefficients of variation (C. V.) were calculated and the mean values plotted against the pH values 5.0 – 7.0.

3.14 Determination of Heavy Metals in Tap water from University of Nigeria, Nsukka (Nkrumah Hostel).

Three serially mixed standard solutions MS_1, MS_2 and MS_3 which contained the metal ions in the following concentrations were prepared:

MS_1: Zn, Cd, Cr, Cu and Fe (1ppm); Pb (2ppm).

MS_2: Zn, Cd, Cr, Cu and Fe (3ppm); Pb (6ppm).

MS_3: Zn, Cd, Cr, Cu and Fe (5ppm); Pb (8ppm).

Into 1 litre standard flasks labeled X_1, X_2, and X_3 were added 1ml of MS_1, MS_2 and MS_3 respectively and made up to the marks with tap water, so that the solutions contained the metal ion spikes as follows:

Zn, Cd, Cr, Cu, Fe (1 – 5 ppb) and Pb (2 – 8 ppb).

The pH of the spiked water samples were adjusted to 7 by careful dropwise addition of dilute HCl or dilute ammonia solution while stirring and monitored using the Digital pH meter type pH_s– 25.

After pH adjustment, 500ml each of the samples were transferred into 1 – litre separating funnels and 25ml of 2% cyanidin chloride solution in 1 – butanol was added to each and shaken for 3 minutes. The phases were allowed to settle before separation.

25ml of 2% cyanidin chloride solution in 1 – butanol was added to the aqueous layer left after extractionand equilibrated for 3mins,allowed to settle and the phases separated. Their respective organic extracts labeled T_1, T_2 and T_3 were employed as standard solutions.

The amount of metals in the spiked water were determined as before using the Unicam 939/959 Atomic Absorption Spectrophotometer against the blank (2% cyanidin chloride in 1 – butanol) and the standard solutions.

3.15 Determination of Heavy Metals in River Water from Opobo River, Ikot Abasi Akwa Ibom, State.

The pH of 500ml volume of river water measured into one-litre separating funnel was adjusted to 7 by dropwise addition of dilute HCl and dilute NH_4OH while stirring and monitored using the Digital pH meter. 25ml of 2% cyanidin chloride solution in 1 – butanol was added, shaken for three minutes and allowed to settle before separation. The process was repeated for two more times (triplicate analysis).

A "metal free" diluent for preparation of standard solutions was obtained by adding 25ml 2% cyanidin chloride in 1 – butanol to each aqueous layer left after extraction. The mixtures were shaken for three minutes, the organic layers discarded and the re-extracted waters were combined. 1.0ml each of MS_1, MS_2, and MS_3 respectively was then made up in 500ml standard flasks with the "metal free" diluent. The pH of each standard solution was adjusted to 7 and 25ml 2% cyanidin chloride solution in 1 – butanol added. The mixture was shaken for three minutes and the phases were allowed to separate. The organic layers were employed again as the standard solutions.

The absorbances of the solutions of the sample extracts were read against those of the blank and standard solutions on the Unicam 939/959 atomic absorption spectrophotometer. The concentrations of each metal in parts per billion (ppb) in the original water samples were obtained directly from a calibration graph plotted after blank corrections.

CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.1 Extraction of Cyanidin Chloride

31.20g of dry dark brown crystals of 3, 3^1, 4^1, 5, 7- pentahydroxyflavylum chloride were obtained from the 400g of *Hisbiscus rosa-sinensis* flowers. This amounted to 7.8% yield.

4.2 Solubility

The solubility of the cyanidin chloride crystals in acetone, methanol, ethanol and 1-butanol were very good compared to hexane, toluene, carbon tetrachloride, diethylether and ethylacetate in which the solubility was either poor or the crystals completely insoluble. The choice for 1-butanol, was based on the fact that others are miscible with water while it is not.

4.3 Spectral Analysis

The characteristic spectra of the aglycon from *Hibiscus rosa-sinensis* obtained from uv/visible spectrophotometric analysis were 279nm and 532nm in the uv and visible regions respectively. In 1-butanol as a solvent,the values were 280nm and 530nm in uv and visible regions respectively before complexation and 455nm and 706nm after complexation with a solution of cadmium ions. The observed spectral shifts of about 176nm is attributed to complex formation through the 3^1,4^1-o-dihydroxy system in the ligand.

Anthocyanidins have two characteristic absorption bands, at 475-560nm (visible region-band 1) and at 275-280nm (ultraviolet region-band 2). Band 1 which is the actual colour band depends on the number and position of the hydroxyl (OH) and methoxy (OME) groups and when they are fixed, the colour then depends on pH and the solvent[20] The aglycon from *Hibiscus rosa-sinensis* exhibited characteristic absorption bands in both the UV and visible regions which compared with that for cyanidin [22]. Results from past workers[23] showed that cyanidin absorbs at about 280nm and 530nm in the UV and visible regions respectively.

4.4 Qualitative Tests for Cyanidins

Table 6: Robinson's qualitative tests for Cyanidins.

Test	Observation
i) Cyanidin Reagent	Rose colour
ii) Oxidation	Fairly stable
iii) colour	Bright blue

From the above colours, and in comparison with the behaviour of anthocyanidin (see table 4) together with the result of the spectral analysis, it could be concluded that the type of anthocyanidin present in *Hibiscus rosa-sinensis* is cyanidin with cyanindiglucoside as the anthocyanin.

4.5 Equilibration Time

The equilibration time required for the extraction of the metals from a mixed standard solution with 2% cyanidin chloride solution in 1-buatnol was investigated at pH 7. The table below is a display of the effect of contact time on the degree of extraction of copper.

Table 7: Quantity of Cu (μg) Remaining in aqueous phase at pH 7 at different time intervals.

Time (Sec)	Cu (II) in Aqueous phase (μg)
30	5.0
60	3.5
90	3.0
120	2.4
150	1.0
180	1.0

Equilibrium is established in three minutes and in subsequent extractions this time was employed.

4.6 Effect of pH Buffer on Extraction

The extraction of Cu, Fe, Cr, Pb, and Zn with 2% 3, 3^{1}, 4^{1}, 5, 7-pentahydroxyflavylium (cyanidin) chloride solution in 1-butanol was studied as a function of pH in the range 1-10.

Table 8: Percent recoveries of some heavy metals in the pH range 1-10

Sample pH	Fe	Cr	Pb	Cu	Zn
pH1	nd	96.20	69.24	nd	22.53
pH2	9.54	98.43	66.48	1.74	16.40
pH3	16.10	96.87	68.43	3.62	15.00
pH4	51.68	99.30	73.06	31.72	20.10
pH5	85.86	99.97	80.88	72.70	21.57
pH6	74.94	96.53	88.73	3.88	15.07
pH7	92.64	98.60	100.00	93.58	83.30
pH8	87.08	93.23	99.76	91.38	85.50
pH9	97.42	81.83	95.11	79.18	93.30
pH10	97.64	81.77	95.06	60.80	85.73

nd = not detected

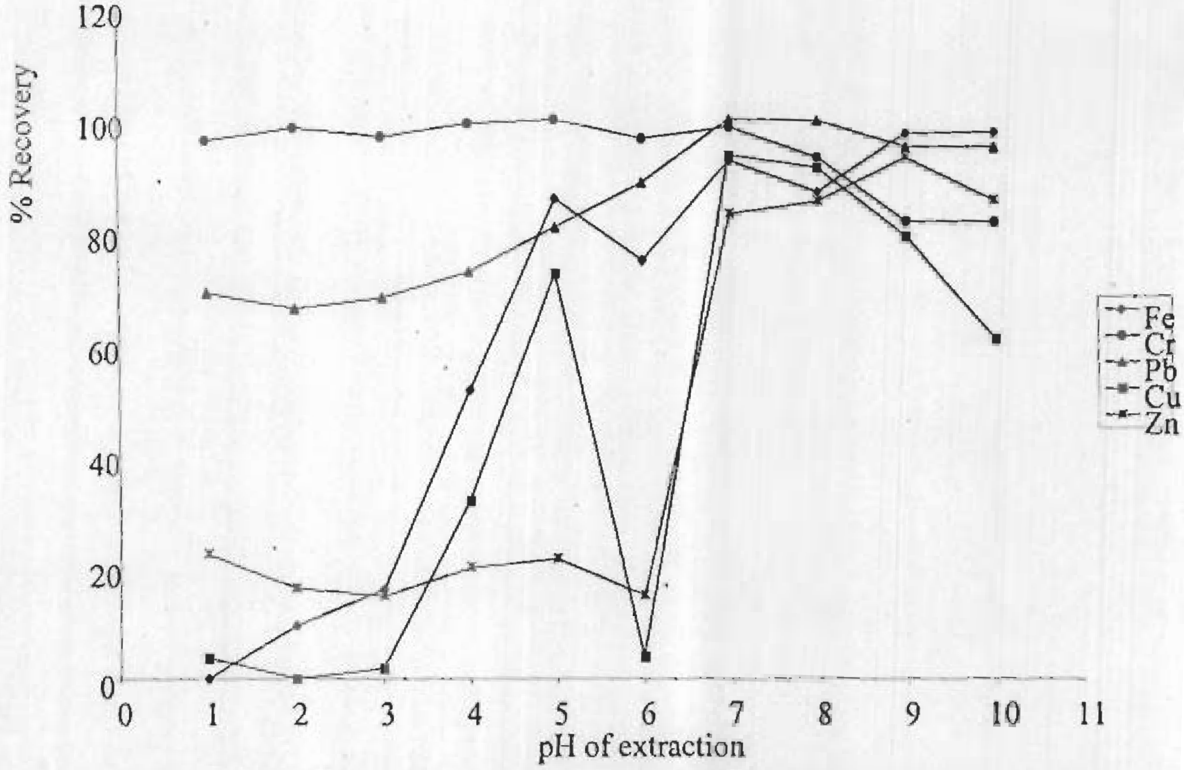


Figure 4.1: Percentage Recovery of Heavy Metals at various pH

Table 8 and figure 4.1 show the effect of pH variations and the extraction of metal ions into the organic phase. It is evident that above 90% of the metals [except Zn (83.3%)] is extracted at pH 7. The result for copper compares favourably with that reported by Agget and Richardson (94.0%) in their study of the use of Bis [4-hydroxypent-2-ylidene] Diaminoethane/Methylisobutylketone as a reagent for copper[76], also a similar percentage (94.3%) was reported by Harries et al77. The quantitative extraction obtained at this pH decreased at pH 8 (except for Zn). The formation of the complexes is due to the behaviour of the ligand as a weak acid leading to the formation of a violet anhydrobase at neutral and high pH which dissociates further as shown in the equation;

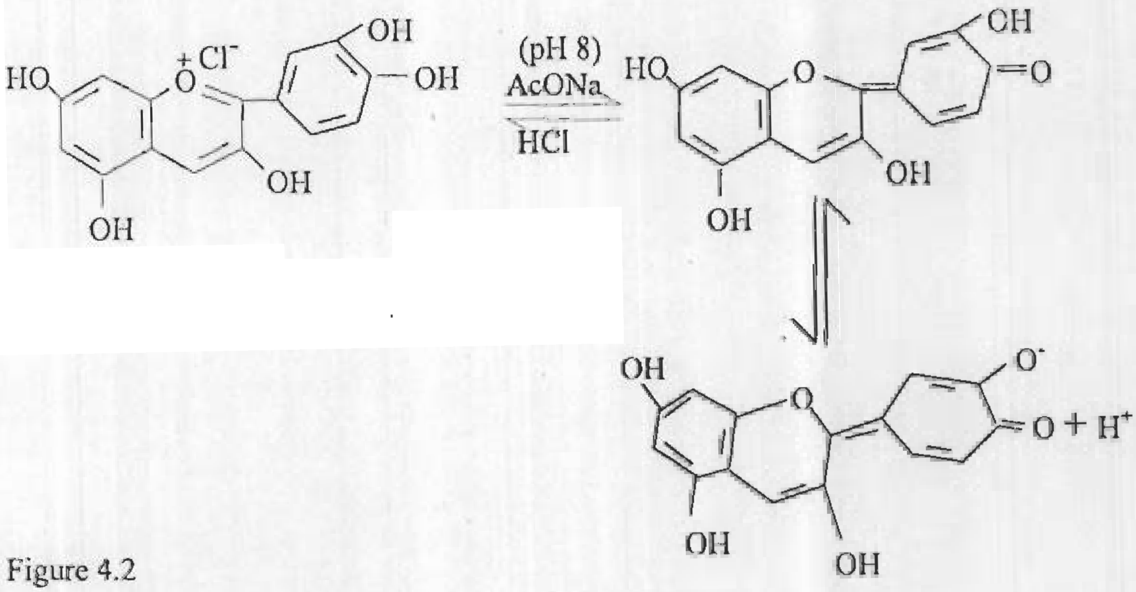
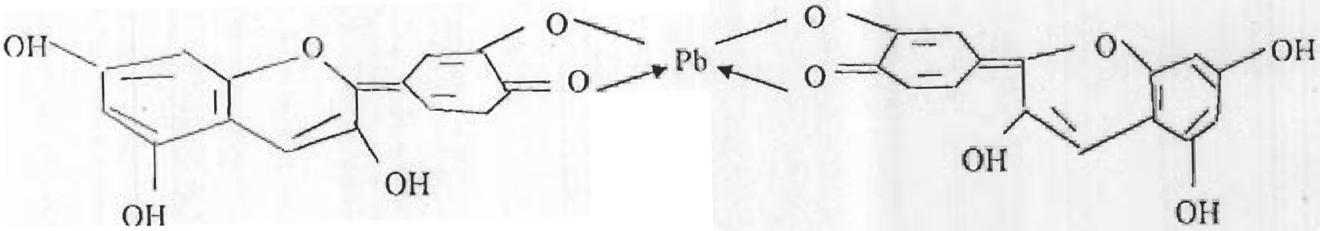


Figure 4.2

Hence forms chelate complexes with metal ions. These complexes are therefore possible because of the presence of the 3,[1] 4[1]-O-dihydroxy system[20] in the ligand. At pH above 9, the percentage extraction diminishes and this may be attributed to the hydrolysis of the metal ions or due to the masking effect of the base component of the buffer[78]. Also according to Everest,[42] cyanidin like other anthocyanidins lack sharp melting point and is unstable at alkaline pH giving blue to green compounds.The very low extraction witnessed between pH 1-4 is due to inability of ligand to dissociate in acid medium, hence less ease of chelate formation.

Since a metal-ligand mole ratio of 1:2 has earlier been suggested,the possible structures of the complexes are as shown in figure 4.3 below:



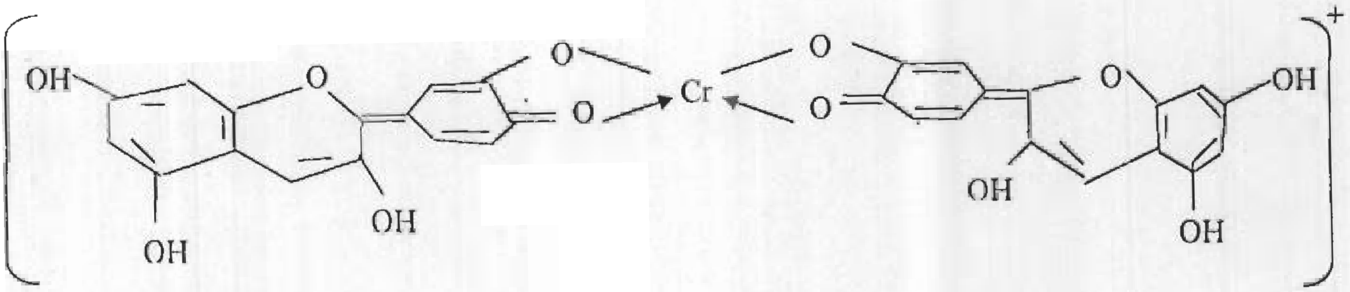


Figure 4.3

4.7 Recovery Experiment At pH Range 5.0-7.0

Since the most quantitative extraction of the metals was observed within the pH range 5.0-7.0, further recovery was restricted within this pH range. The results Table 9 and figure 4.4 revealed an optimum pH of 7 hence all other work using 3, 3^1, 4^1, 5, 7-pentahydroxyflavylium chloride was done at this pH (7).

Table 9: Mean Percent Recoveries of Some Trace Heavy Metals at pH Range of 5.0- 7.0

METAL/pH	5.0	5.5	6.0	6.5	7.0
Zn(mean ± sd)	14.39 ± 1.89	19.51±1.96	21.10±1.99	84.59±3.34	86.52±3.20
C.V.%	13.13	10.05	9.43	3.95	3.70
Cd (mean ±sd)	4.35 ±0.25	62.66 ±2.70	70.24 ±2.78	83.27 ±2.90	88.22 ±3.00
C.V%	5.75	4.31	3.96	3.48	3.40
Cr (mean ±sd)	96.08 ±2.37	92.78 ±2.29	89.55 ±2.24	94.79 ±2.35	95.49 ±2.35
C.V%	2.47	2.47	2.50	2.48	2.46
Cu (mean ±sd)	3.69 ±0.18	30.22 ±1.56	60.81 ±22.11	89.32 ±2.14	91.50 ±2.17
C.V%	4.88	5.16	36.36	2.40	2.37
Fe (mean ±sd)	51.55 ±2.37	75.21 ±2.88	86.42 ±3.12	87.74 ±2.98	93.87 ±3.42
C.V%	4.60	3.38	3.61	3.40	3.64
Pb (mean ±sd)	70.65 ±2.19	78.35 ±2.34	86.20 ±2.25	96.89 ±2.65	97.08 ±2.66
C.V%	3.10	2.99	2.61	2.74	2.74

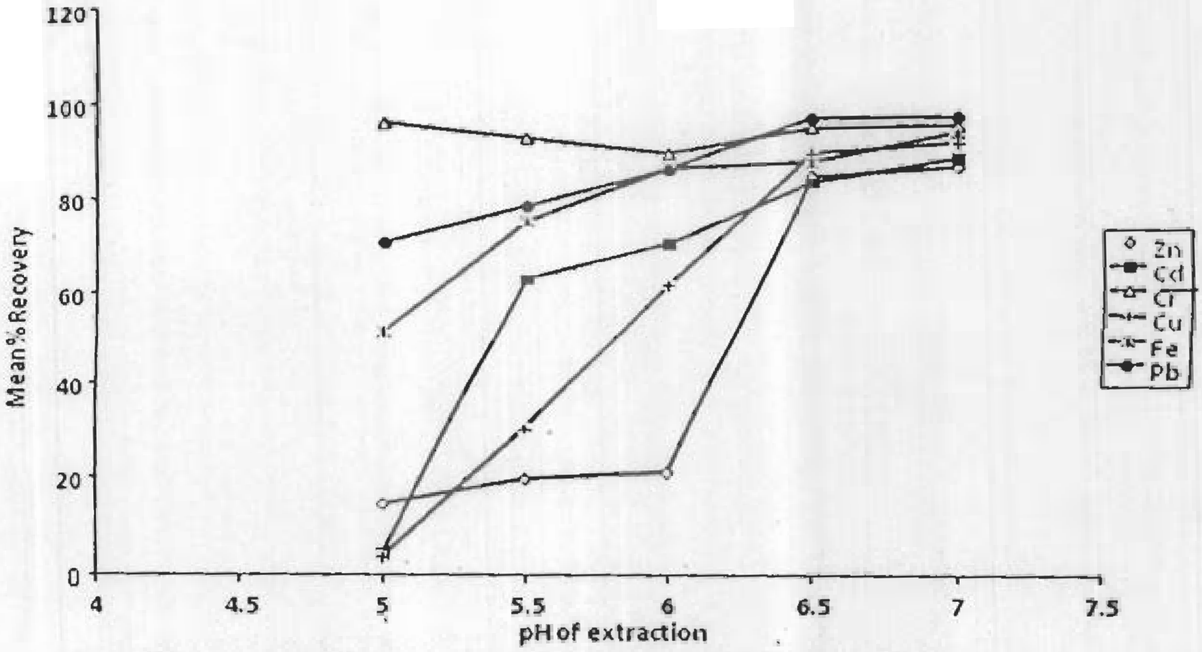


Figure 4.4: Mean Percent Recoveries of Heavy Metals at pH 5.0 – 7.0

4.8 Heavy Metals in Tap water and River water

Data on the one-step pre-concentration of six heavy metals (Cd, Cu, Cr, Fe, Pb and Zn) from spiked tap water and river water at pH 7 are presented in tables 11 and 12.

Table 11: Percentage Extraction of Metals from Spiked Tap Water

Metal	Percentage Extraction
Zn (mean ± sd)	115.23± 1.38
C.V. %	1.2
Cd (mean ± sd)	101.44± 3.35
C.V. %	3.3
Cr (mean ± sd)	102.03± 3.98
C.V. %	3.9
Cu (mean ± sd)	117.94± 5.54
C.V. %	4.7
Fe (mean ± sd)	149.73± 3.74
C.V. %	2.5
Pb (mean ± sd)	103.33± 2.69
C.V. %	2.6

Table 12: Concentrations of the Metals determined in Water of Opobo River, Ikot Abasi, Akwa Ibom (µg/l)

METAL	B_1	B_2	B_3	Mean ±sd C.V.%
Zn	5.02	5.48	5.31	5.27 ±0.23 4.4
Cd	2.81	3.02	3.03	2.95 ±0.12 4.1
Cr	0.12	0.08	0.14	0.11 ±0.03 27.3
Cu	15.21	15.62	14.01	14.95± 0.84 5.6
Fe	30.08	31.61	30.74	30.81 ± 0.77 2.5
Pb	10.84	11.21	10.49	10.85± 0.36 3.3

The mean percentage recoveries (n=3) ranged from 101.44% for cadmium to 149.73% for iron in tap water while the mean recoveries in the river water ranged from 0.11 µg/l for chromium to 30.81 µg/l. The coefficients of variation (%) at pH 7 were as follows; Zn (1.2), Fe (2.5), Pb (2.6), Cd (3.3), Cu (4.7) and Cr (3.9) for tap water and Fe (2.5), Pb (3.3), Cd (4.1), Zn (4.4), Cu (5.6) and Cr (27.3) for the river water. The reproducibilities were good.

Table 13: Table for the Standard Graph of Copper

OUT PUT	CONCENTRATION (ppb)
0.068	1
0.134	2
0.148	3
0.187	4
0.189	5
0.199	6
0.261	7
0.323	8

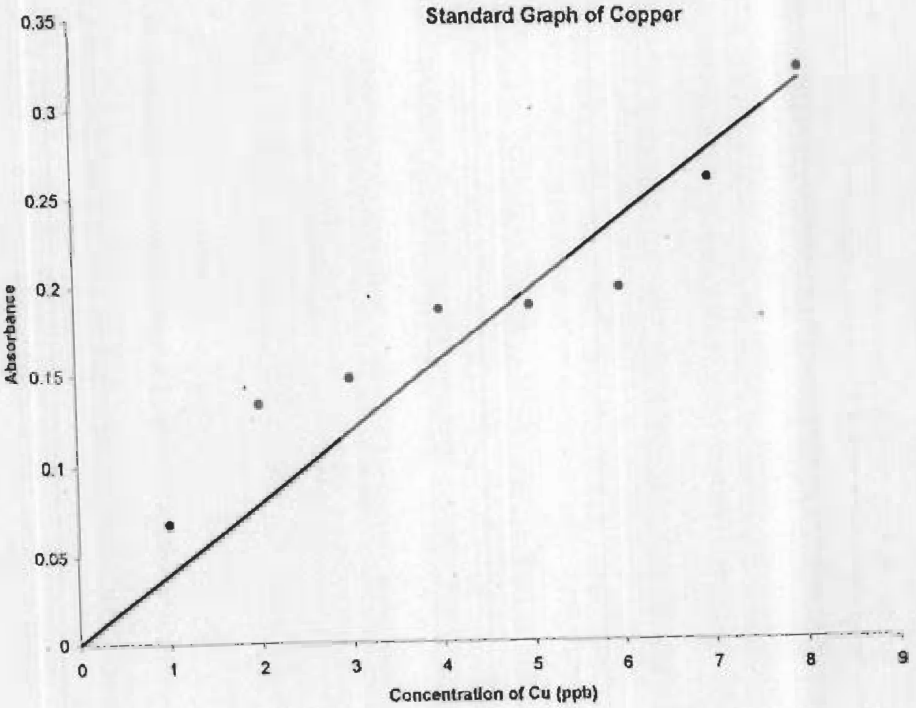


Figure 4.5

Table 14: Table for the Standard Graph of Zinc

OUT PUT	CONCENTRATION (ppb)
0.001	1
0.004	2
0.007	3
0.009	4
0.012	5
0.016	6
0.020	7
0.024	8

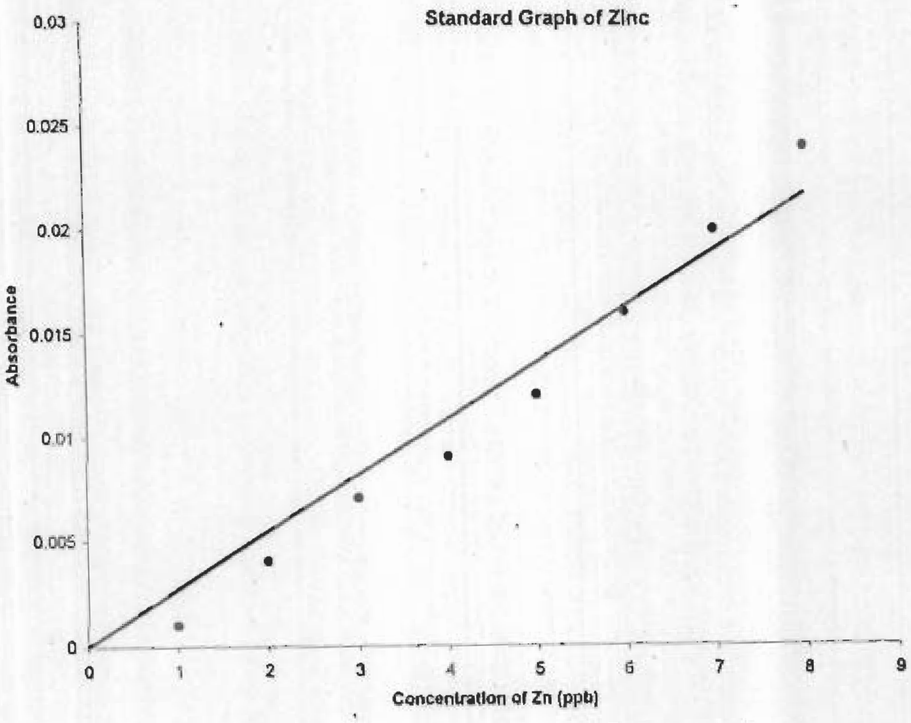


Figure 4.6

Table 15: Table for the Standard Graph of Cadmium

OUT PUT	CONCENTRATION (ppb)
0.010	I
0.230	2
0.270	3
0.380	4
0.530	5
0.625	6
0.720	7
0.805	8
0.890	9

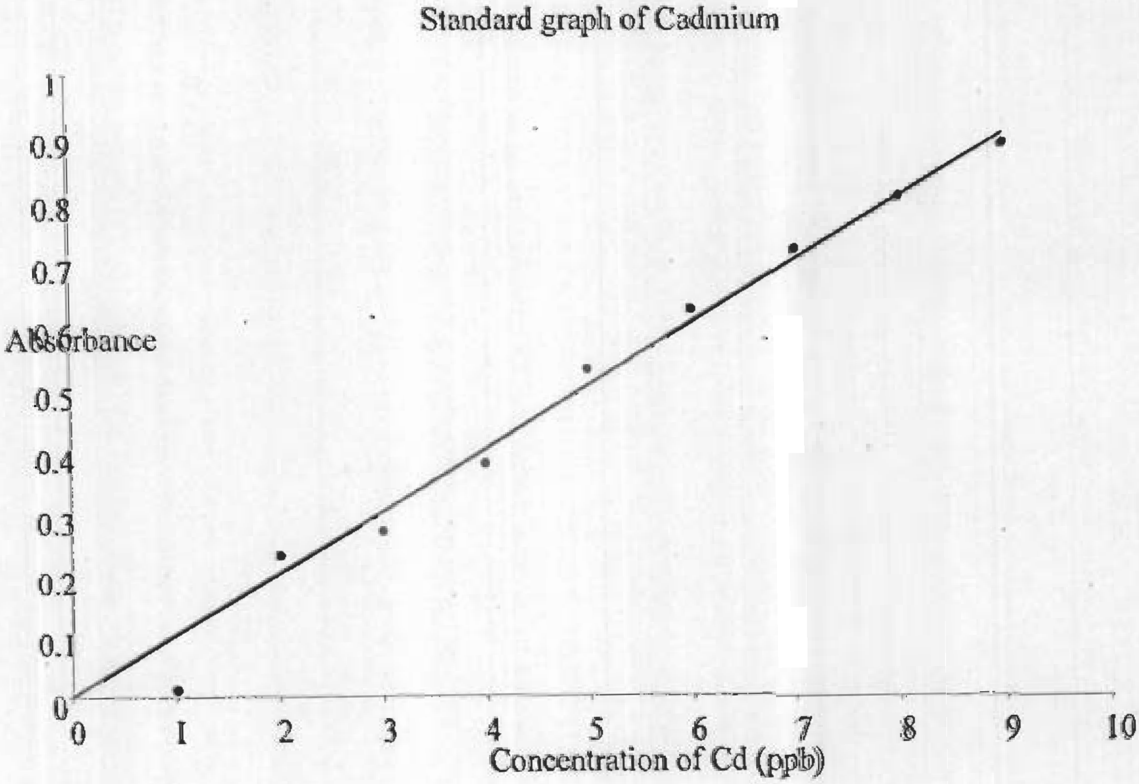


Figure 4.7

Table 16: Table for the Standard Graph of Chromium

OUT PUT	CONCENTRATION (ppb)
0.010	1
0.019	2
0.029	3
0.039	4
0.049	5
0.062	6
0.072	7
0.095	8

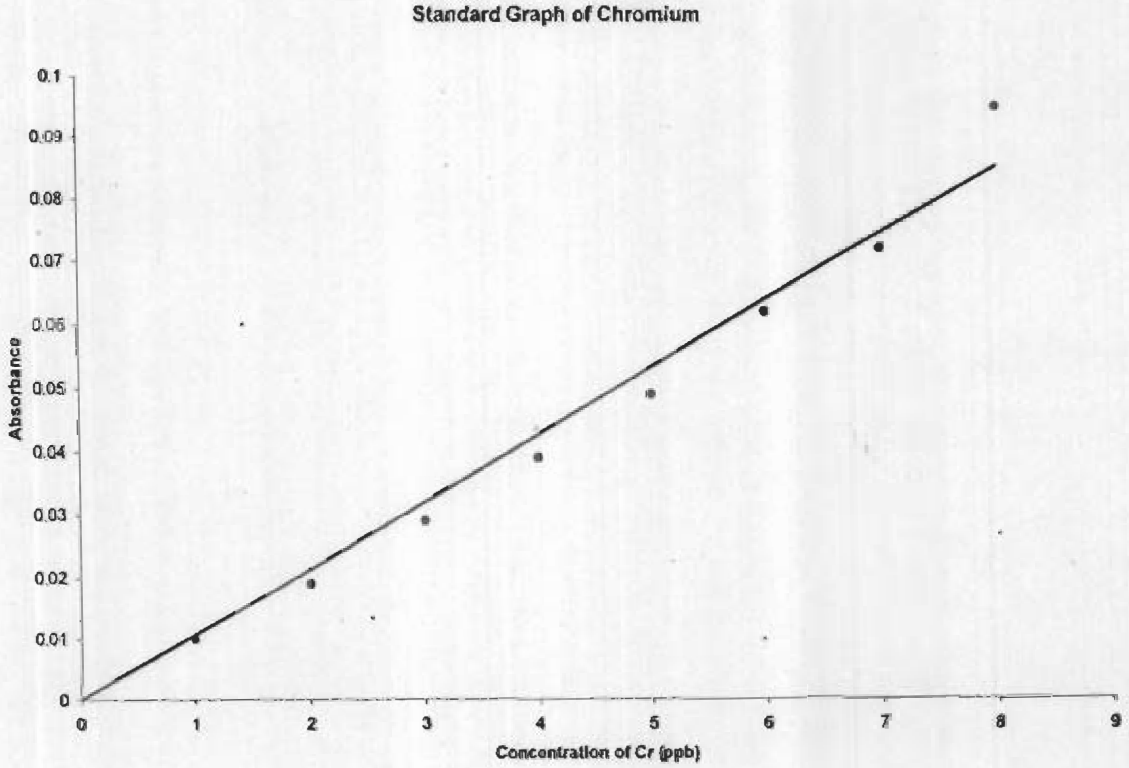


Figure 4.8

Table 17: Table for the Standard Graph of Lead

OUT PUT	CONCENTRATION (ppb)
0.003	1
0.004	2
0.005	3
0.006	4
0.008	5
0.009	6
0.010	7

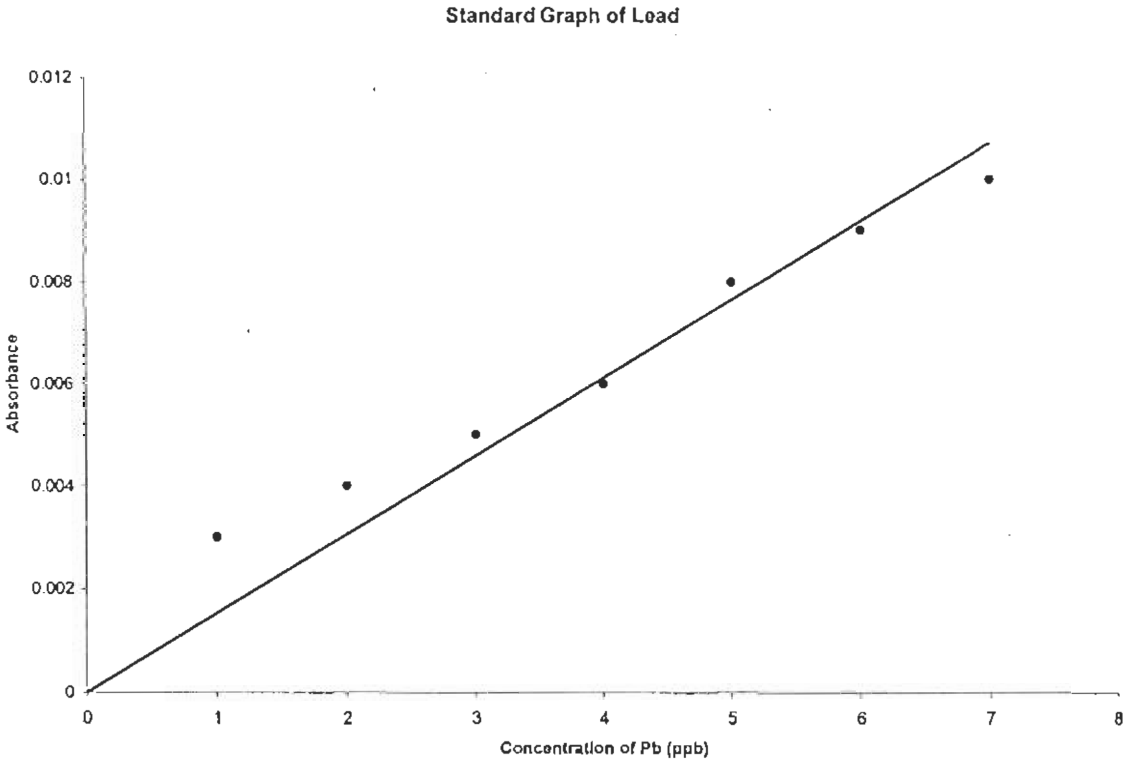


Figure 4.9

Table 18: Table for the Standard Graph of Iron

OUT PUT	CONCENTRATION (ppb)
0.005	1
0.017	2
0.027	3
0.028	4
0.038	5
0.040	6
0.047	7
0.054	8
0.066	9
0.085	10

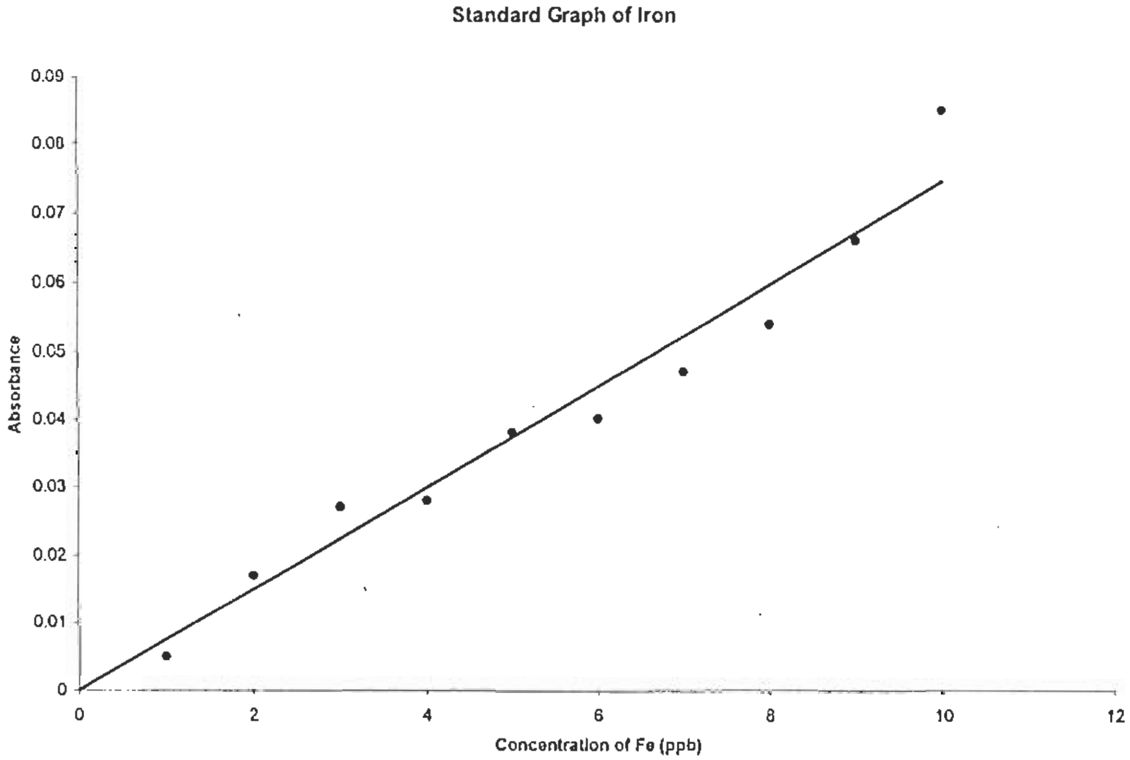


Figure 4.10

The overall mean levels of the heavy metals in both tap-water and river water were compared with Environmental Protection Agency (EPA) maximum allowable concentrations in Table 19 and World Health Organization (WHO) standard for drinking water Table 20.

Table 19: EPA Maximum Allowable levels compared with both Tap water and River water

Metal	Tap water Overall mean (μg/l)	Riverwater overall mean (μg/l)	EPA maxima (μg/l)	
			Marine	Public water
Cd	1.44	2.95	10	10
Pb	3.33	10.85	50	50
Cr	2.03	0.11	100	50
Fe	149.73	30.81	300	300
Cu	117.94	14.95	50	1000
Zn	15.23	5.27	100	5000

Source EPA (1972); 1976 [79, 80]

Table 20: WHO Standard Values for Drinking Water

Parameter	**Ingest Desirable level**	**Maximum permissible**
Cadmium (mg/l)	—	0.01
Chromium (mg/l)	—	0.05
Iron (mg/l)	0.1	1.0
Lead (mg/l)	—	0.05
Zinc (mg/l)	5.0	15.0
Copper (mg/l)	0.05	1.6

Source: International Standard for Drinking Water WHO, 1973[81]

In both the tap water and river water, iron has the maximum concentrations while cadmium was least in tap water (mean value 1.44μg/l) and chromium was least in river water (mean value 0.11μg/l).

On the whole, the levels of heavy metals studied in both the tap water and river water can be judged not to constistute any danger at the moment since their values are well below the EPA and WHO maxima.

CONCLUSION

3, 3^{1}, 4^{1}, 5, 7-pentahydroxyflavylium chloride (cyanidin chloride) was obtained from *Hibiscus* flowers. The extraction process was inexpensive, efficient, fast and its solubility in 1- butanol was equally very good. The spectra of the aglycone from *H*ibiscus rosa-sinensis obtained from UV/Visible spectrophotometric analysis were 279nm and 532nm in the UV and visible regions respectively while results from past workers showed that cyanidin absorbs at about 280nm in UV region and at about 530nm in the visible region. Hence the UV/Visible spectra were in agreement with literature.

Solvent extraction studies on Cu, Cd, Cr, Fe, Pb and Zn showed that about 90% of the metals is extracted in the pH range 5-9 with pH 7 as the optimum. Complex formation with the heavy metals was confirmed from spectrophotometric analysis of both free ligand and the complexed ligand and the sensitivity of the extraction was enhanced through pH adjustment. Results of the chelation of Cd, Cu, Cr, Fe, Pb and Zn from tap water and river water show that these water sources are not significantly contaminated with these heavy metals. On the whole, the levels of these heavy metals studied in both water sources can be judged not to present any danger at the moment since their values are well below the EPA and WHO maxima.

Considering the cheapness and ready availability of this reagent over the conventional spectrophotometric reagents, it could be very useful as an analytical reagent.

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