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RESEARCH ARTICLE

METAL ION INTERACTION WITH ELECTRON RICH SPECIES

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ABSTRACT

A description of the transition metal ions especially copper(II), iron(III), uranium(VI) and thorium(IV) based on the toxicity of the elements on human organs is included. A brief description of the electron rich species interacting with copper (II), iron(III), uranium(\VI) and thonum(IV) metal ions is also included.

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INTRODUCTION

Electron rich species are compounds which have generally nitrogen, sulphur, and oxygen donor atoms. These atoms are acting as basic centres for interaction with metal ions. These compounds are natural and synthetic. Several of these compounds have been found biologically active and used as medicine. Many others are useful in industry. In the hetrocyclic compounds the donor atoms are present in the ring, side chain and or directly attached to the carbon atom of the ring. Interaction of transition metal ions with electron rich species results in the formation of complexes. Transition metal complexes have been used for carcinostatic activity, antitumour activity, fungicide and insecticide. Many sulphur and nitrogen containing compounds viz pyridine-2-carboxaldehyde thiosemicarbazone (1) and isoquinoline-1-carboxaldehyde thiosemicarbazone (2) have been found to have a broad range of anticancer activity [1]. Metal ion interaction with vitamins, enzymes and nucleic acid leads to the formation of chelate. These metal chelate on reacting to the normal cell replace the essential metal ions from the normal enzyme system, thus, metabolic activity is changed. The anticancer drug may deactivate the carcinogenesis by binding the normal metal ion [2-7].

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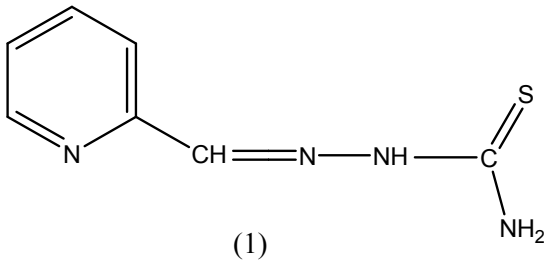

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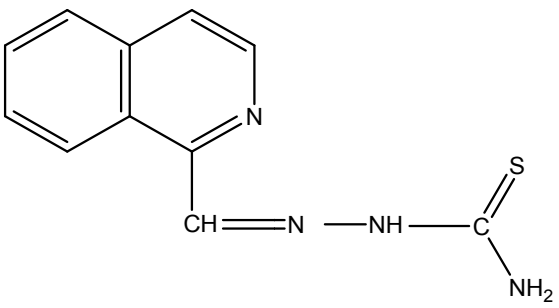
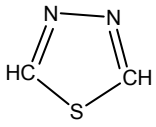


(2)

Many metal chelates of folic acid [8], riboflavine [9], adenine [10] and thioguanine [11] have also been reported. Bismuthiol-1 is a five membered hetrocyclic ring system having three suphur and two nitrogen atoms and a derivative of 1,3,4 thiadiazole (3) in which hydrogen atom attached to carbon atom at 2 and 5 positions are replaced by –S-H group (4).



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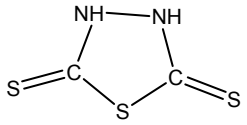
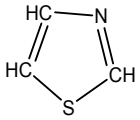


(4)

Bismuthiol-1 (2,5-dimercapto 1,3,4 thiadiazole) is an important analytical reagent for bismuth [12]. Several metal complexes of bismuthiol-1 with metal ions such as Zn(II), Cu(II), Ni(II) and Co(II) have been prepared [13]. It is also used as a reagent for spectrophotometric determination of palladium [14-16]. Uranyl (UO_2^{2+}) and thorium(IV) givechelate after interaction with bismuthiol-1 in aqueous methanol mixture [17]. Other heterocyclic compounds with nitrogen and sulphur coordinating sites like thiazole (5), and 2 acetamido-5 nitro thiazole (6) are also biologically active and form complexes with UO_2^{2+} and Th^{4+} ions. Thiazole, imidazolethiol on interaction with metal ions form biologically active complexes acting as fungicides. Many other complexes are used as anti oxidant [13,18,19].



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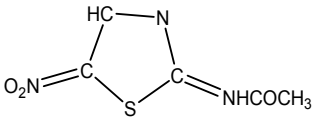
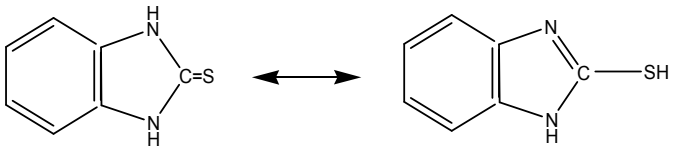
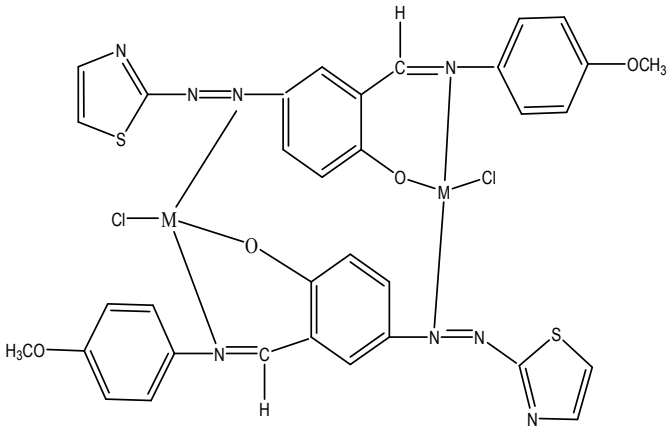


(6)

2-Benzimidazolethiol is an industrially important compound. It has nitrogen and sulphur basic centers for interaction with metal ions. It shows tautomerisms.

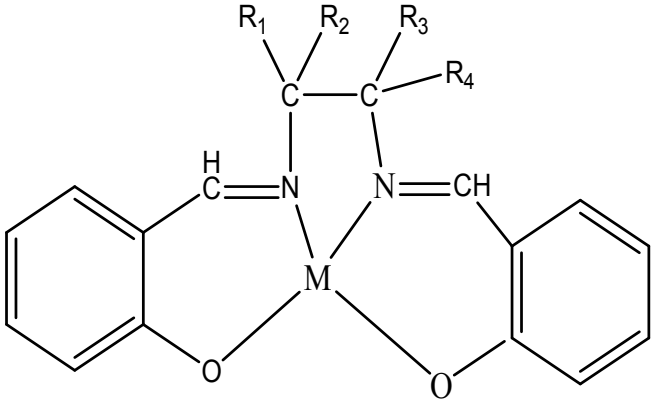


It has good coordinating ability. Certain metallic salt of 2-benzimidazole thiol have been used as heat stabilizer for polyamide. It is specific reagent for determination of various metals. Spectrophotometric determination of Pd(II), Ru(III) have been done using this compound [20]. Adduct formation with Sn(II), Sn(IV) and Sb(III) have been reported [21]. Interaction of inner tranisition metal ions such as Y(III), Pr(III), Nd(III), Sm(III), Gd(II) and Dy(III) with 2-benzimidazole thiol led to the formation of 1:1 complexes in aqueous solution [22]. Schiff bases are an important class of compounds in medicinal and pharmaceuticals fields. They show biological applications including antibacterial [23-25], antifungal [26,27], and antitumour activities [28,29]. Many of researchers studied the synthesis, characterization and structure-activity relationship of Schiff bases [30, 31]. Schiff base complexes with transition metals have also played a prominent role in the development of coordination chemistry [32]. Transition metal complexes of Schiff bases have been amongst the most widely studied coordination compounds, since they are found to be useful as biochemical, analytical and antimicrobial reagents [33-35]. A series of complexes [36] involving Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg (II) ions and Schiff's base ligand obtained by condensation of 5-(2'-thiazolylazo) salicyaldehyde and p-methoxy aniline have been reported [36]. The structure of the complexes may be represented as:



where M= Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II).

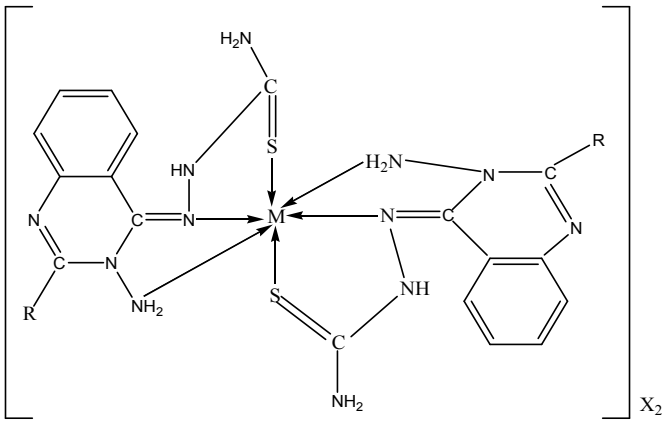
The tetra dentate Schiff bases are interesting complexing reagents [37-41] because they form highly stable metal chelate compounds with a limited number of metal ions such as Cu(II), Ni(II), Co(II), Co(III), Fe(II), Fe(III), Pd(II), Pt(III), VO(II), UO_2(II). The metal chelates are extractable in organic solvent. The reagents bis(salicylaldehyde) tetramethylethylenediimine (H_2SA_2Ten), bis(salicylaldehyde ethylenediimine (H_2SA_2en), and bis(salicylaldehyde) propylenediimine (H_2SA_2Pn) have been reported for the determination of uranium, iron, copper and nickel in mineral ore samples and phosphate rock residues [42-43]. Phenyl group substituted ligands such as bis(salicylaldehyde)-dl-stilbenediimine (dl-H_2SA_2S) and bis(salicylaldehyde)-meso-stilbene-diimine (meso- H_2SA_2S) have been reported for HPLC determination of uranium, iron, copper and nickel [44]. The structure of these complexes, H_2SA_2Ten, H_2SA_2en, H_2SA_2Pn, dl-H_2SA_2S and meso- H_2SA_2S is shown below:



Where H_2SA_2en ;R_1, R_2, R_3 and R_4 = H
H_2SA_2Pn ; R_1 =CH_3, R_2, R_3 and R_4 = H
H_2SA_2Ten ; R_1, R_2, R_3 and R_4 = CH_3
dl-H_2SA_2S ; R_1 and R_3 = C_6H_5, R_2 and R_4 = H
meso- H_2SA_2S ; R_1 and R_3 = C_6H_5, R_2 and R_4 = H
M= Cu(II), Fe(III), Ni(II), UO_2(II).

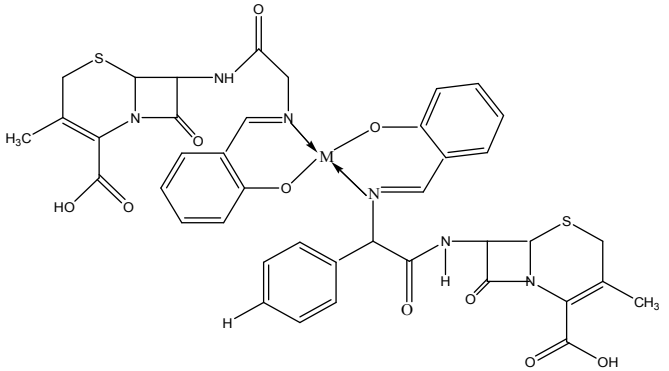
The chemistry of thiosemicarbazones has received considerable attention in view of their variable bonding modes, promising biological implications, structural diversity and ion-sensing ability [45,46]. They have been used as drugs and are reported to possess a wide variety of biological activities against bacteria, fungi and certain type of tumours and they are also useful models for bioinorganic processes [47]. The

inhibitory action is attributed due to their chelating properties [48-50]. The activity of these compounds is strongly dependent on the nature of the hetero atomic ring and the position of attachment to the ring as well as the form of thiosemicarbazone moiety [51]. These are studied extensively due to their flexibility, their selectivity and sensitivity towards the central metal atoms, and similarities with natural biological substances due to the presence of imine group (-N=CH-) which imparts the biological activity [52]. Copper(II) complexes of thiosemicarbazones are good antimicrobial agents [53]. It has been reported that copper(II) chelates of anthracene-9-carboxyaldehyde thiosemicarbazones [54] exhibited antitumour activities whereas copper(II) complexes of phenylglyoxal bis(thiosemicarbazone) showed cytotoxic activity [55]. Complexes of Co(II), Ni(II) and Cu(II) have been synthesized with 3-amino-2-phenyl 4(3H) quinazoline thiosemicarbazone (APQT). The complexes have been formulated as $[M(APQT)_2]X_2$ where M= Co(II), Ni(II) and Cu(II); X= Cl^-, Br^-,I^-,NO_3^- and ClO_4^- [56]. The ligand APQT acts as a neutral tridentate ligand and coordination takes place through azomethine N, thione S and primary amino group of quinazoline. The geometry of these complexes is octahedral in nature and shown as:



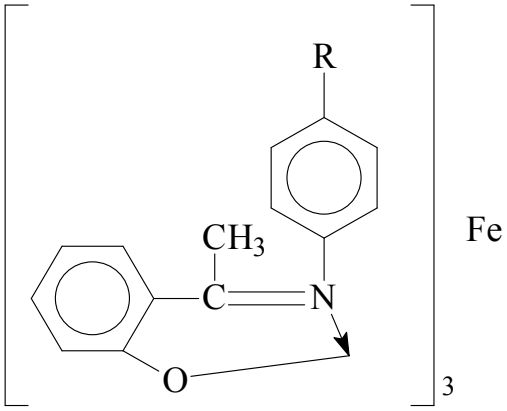
Where R=Phenyl

Cephalexin belongs to the first generation cephalosporins. Many drugs possess modified toxicological and pharmacological properties when they are complexed [57,58]. In this series, metal complexes of Cu(II) and Zn(II) with Schiff base of cephalexin (Salicylidene cephalexin) of the type, ML_2, have been prepared [59]. The conductometric titration revealed that one mole of metal complexed with two moles of ligand. The physical, analytical and spectral studies of Schiff base and its complexes confirmed that the coordination of metal to the Schiff base occurred through phenolic deprotonated oxygen and the imino nitrogen. The structure of the complexes is shown below.



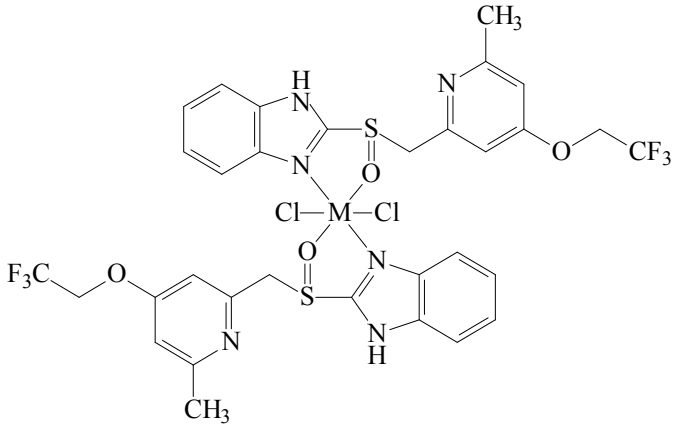
Where M=Cu(II) / Zn(II).

Makkar and coworkers [60] have reported the synthesis of Fe(III) complexes of 1-(2'-hydroxyphenyl) ethylideneanilines. The structures of these complexes were proposed on the basis of elemental analysis and IR studies. The metal to ligand stoichiometry was found to be 1:3 and the possible structureis shown as:



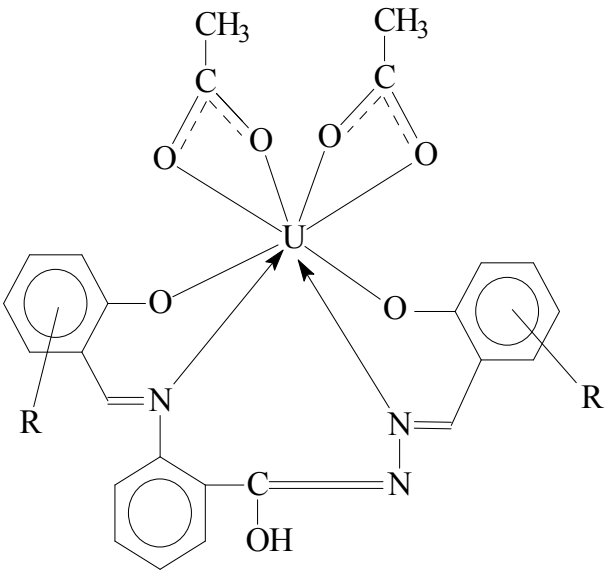
Where R = H, – CH_3, – OCH_3, – OC_2H_5, Cl, Br, OH

A simple spectrophotometric method has been reported [61] for the determination of Fe(III) based as a thiocyanato mixed complex with N-hydroxy N(-p-chloro)-phenyl, N'-p- tolyl-m-toluamidine. The red-orange complex is quantitatively extracted into benzene. The benzene extract showed an intense peak at 465 nm with molar absorptivity of 1.34×10^4 L $mol^{-1}cm^{-1}$. Beer's law was obeyed in the concentration range of 0.2 to 4.2 ppm. It was reported in the literature [62] that lansoprazole, an antiulcer drug, was found to react with Cu(II), Co(II), Ni(II), Hg (II), Zn(II) and Cd(II) resulting in the formation of complexes. The structure of these complexes is shown below:

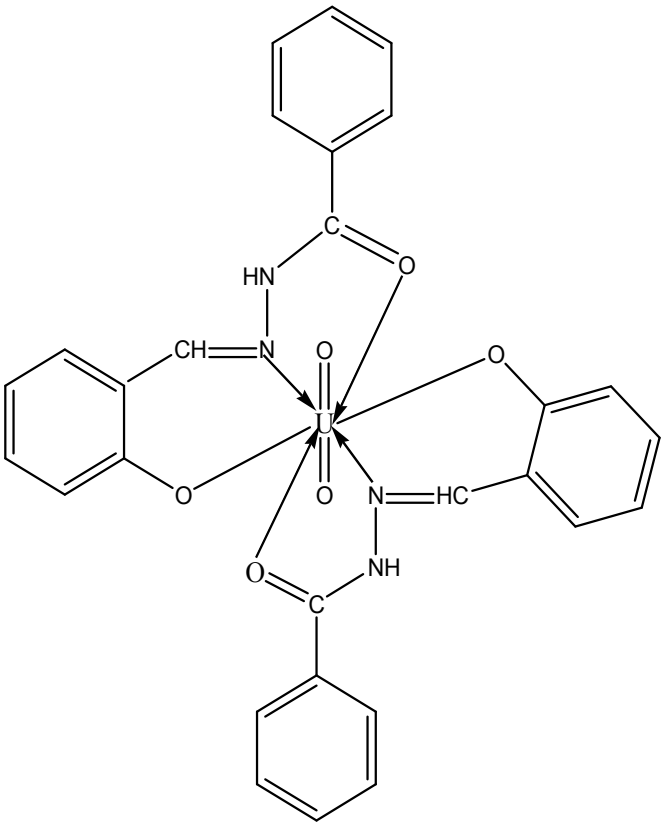


Where M = Cu(II), Ni(II), Co(II), Hg (II), Zn(II) and Cd(II)

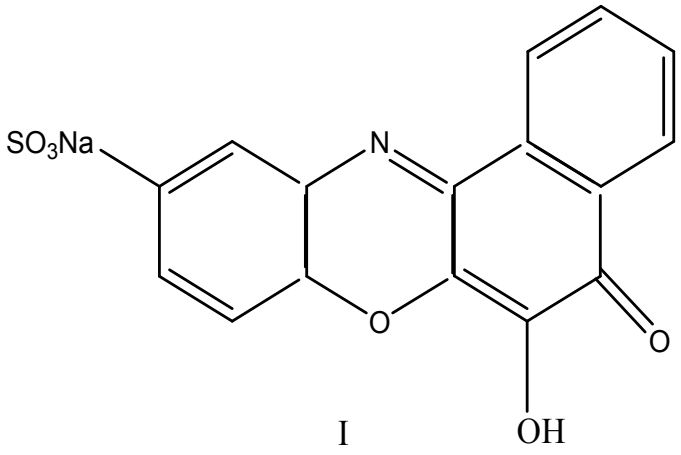
Metal-ligand stability constants fall in the order of Cu(II) > Co(II) ≈ Ni(II); Hg(II) > Zn(II) > Cd(II). There has been a growing interest in the synthesis of uranium(IV) complexes [63-66]. As a further contribution to this area, Schiff base complexes of 2-aminiobenzoyl hydrazones with uranium(IV) acetate have been synthesized [67]. These complexes were characterized on the basis of elemental analysis, conductivity, magnetic and infrared spectral data and hence the following structure was proposed.



The environment around uranium(IV) consists of six oxygen and two nitrogen atoms. Of the six oxygens, four belongto two acetate moieties. The uranium atom is likely to be above the plane of the ligand-coordinating atoms. The interaction of uranium(VI) with hydrazones led to the formation of coloured complexes. These reactions have been studied spectrophotometrically [68,69]. Biradar and Angadi [70] have reported the synthesis and spectral studies of uranium(VI) complexes with aroylhydrazones. The resulting reddish brown complexes are soluble in N,N-dimethyl formamide and dimethyl sulphoxide. These complexes have 1:2 stoichiometry and can be represented as:



The coordination properties [71] of five dyes of alizarin green series, Viz., compounds I-V, with uranyl ions were studied in the presence of cationoid surfactants (cetylpyridinium bromide, carbethoxypentadecyltrimethyl ammonium bromide).



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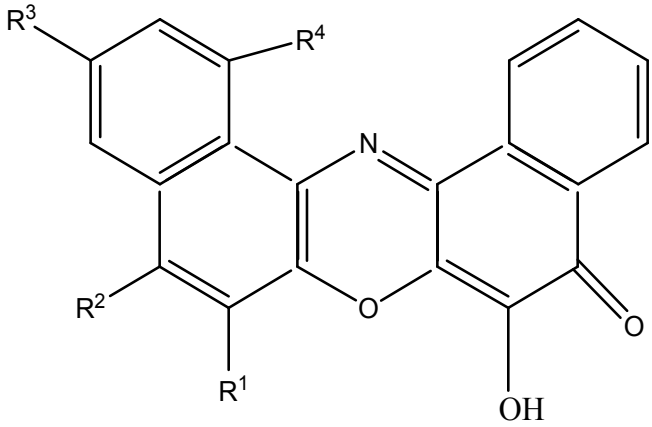
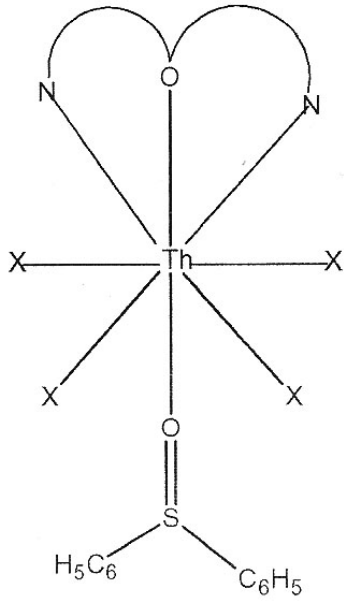


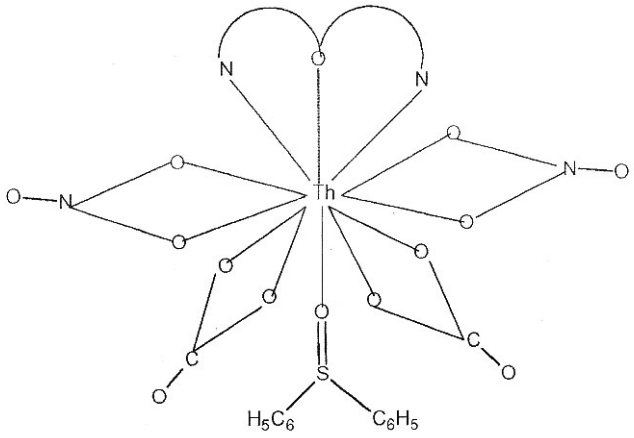
II, $R^2 = SO_3Na$, $R^1 = R^3 = R^4 = H$
III, $R^2 = R^3 = SO_3Na$, $R^1 = R^4 = H$
IV, $R^3 = R^4 = SO_3Na$, $R^1 = R^2 = H$
V, $R^1 = R^3 = SO_3Na$, $R^2 = R^4 = H$

Ternary complexes of the composition $UO_2L_2S_2$, $UO_2L_2S_4$, $UO_2L_2S_6$ and, $UO_2L_3S_9$ where L is dye and S is surfactant are formed in weekly acid solutions. The formation constants of the complexes were established at pH 4.3-4.8,4.4-4.9 and 5.2-5.9 for dyes II, III and IV, respectively. The stability of the complexes is sufficient to enable a direct photometric determination of uranium in excess dye and surfactant. Th(IV) chemistry presents an excellent area of research because of its possibility of formation of compounds with high coordination number. Th(IV) with ionic radius of 0.99Å and a charge of 4+ fulfills the optimum conditions required for a high coordination.

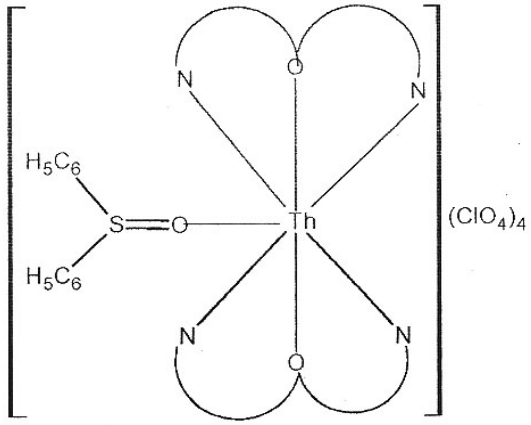
The coordination chemistry of thorium(IV) has been reviewed [72-74]. Agarwal and Prasad [75] have reported the synthesis of some mixed ligand complexes of thorium (IV) derived from 4[N–(2'–hydroxy–1'–naphthalidene) amino] antipyrene semicarbazone (HNAAPS) or 4[N–(Cinnamalidene) amino] antipyrine semicarbazone (CAAPS) as primary ligand and diphenyl sulfoxide (DPSO) as secondary ligand with the general composition $ThX_4.n(L).DPSO$ (n=1, X = Cl, Br, NCS or NO_3^-; n = 2, X = 1 or ClO_4, L = HNAAPS or CAAPS). The infrared studies revealed that semicarbazone behaves as neutral tridentate (N, N, O) while DPSO coordinates through its oxygen atom. The nitrates are bicovalently bonded, while thiocyanate are N-coordinated in these compounds. The structures of thorium(IV) complexes are given below:



$[ThX_4(L)DPSO]$; X = Cl, Br or NCS; L = HNAAPS or CAAPS

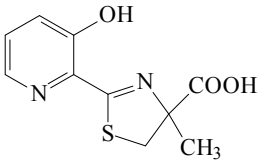


$[Th(NO_3)_4\ (L)DPSO]$; L = HNAAPS or CAAPS

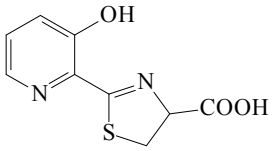


$[Th(L)_2DPSO](ClO_4)_4$; L = HNAAPS or CAAPS

The complexation of Th(IV) with desmethyldesferrithiocin (H_2DMDFT), a derivative of siderophore desferrithiocin (H_2DFT) was studied [76] by potentiometry, spectrophotometry and NMR. Three protonation constants of the ligand were determined by potentiometric titrations and ^{1}HNMR and assigned to the phenolate group, the nitrogen at the hydroxypyridine ring and the carboxylate group. The spectrophotometric studies suggested a 1:2 Th/ desmethylferrithiocin complex. The formation constant of 1:2 complex, Th $(DMDFT)_2$, was determined (log β_2 = 26.7).



H_2DFT



H_2DMDFT

The interaction of Th(IV) with N-hydroxy ethylethylenediaminetriacetic acid (HEDTA) and with the related ligands nitrilotriacetic acid (NTA), N-hydroxyethyliminodiacetic acid (HIMDA), N-methyliminodiacetic acid (MIMDA), oxybis (ethylenenitrile) tetraacetic acid (OETA) and ethylenebis (oxyethylenenitrile) tetraacetic acid (EOTA) has been studied potentiometrically [77]. Evidence is presented for hydrolysis reactions of Th(IV) chelates of all these ligands as the pH increased. The equations for the hydrolysis reactions are presented as:

$$Th\ L\ (H_2O)^+ \underset{}{\overset{K_a}{\rightleftharpoons}} Th\ (OH)\ L + H^+$$

$$2\ Th\ L\ (H_2O)^+ \underset{}{\overset{K_D}{\rightleftharpoons}} Th\ L(OH)_2\ Th\ L + 2H^+$$

$$2\ Th\ (OH)\ L \underset{}{\overset{K_d}{\rightleftharpoons}} Th\ (OH)_2\ Th\ L$$

The formation constant of 1:1 Th(IV) – HEDTA chelate was determined to be $10^{18.5}$. It was found that Th(IV) reacts with 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone [78,79] to yield a stable complex. This reagent is used mainly for separation of Th(IV) from other metals to determine its content in rock and soil.

(i) Interaction of Fe(III) with piroxicam. The resulting coloured complex has been utilized for spectrophotometric determination of Fe(III) in soil samples.
(ii) Interaction of Cu(II) with cefixime. The Cu(II)-cefixime complex has been studied spectrophotometrically to determine the Cu(II) content in water samples.
(iii) Interaction of Th(IV) with rifampicin resulted in the formation of coloured complex. A spectrophotometric method was developed to determine the Th(IV) in soil samples.
(iv) Interaction of U(VI) with rifampicin. The U(VI)-rifampicin complex was exploited to develop a spectrophotometric method for determination of U(VI) in soil samples.

The use of metals in human history has yielded great benefits as well as unexpected harmful consequences. The generic term metal refers to roughly70electropositive elements in the periodic table. As agroup, they share some common physical, chemical, and electrical properties. Further, while sharing

common properties, metals exhibit wide ranges with respectto one another, in both chemical behaviour and the measured values of those common properties. Historically, it has been the exploitation of these properties of metals which has led to successive waves of progress in the development of modern technological society and its dependence on, and increasing appetite for metals. The chemical and radioactive behaviour of some metals affects the biological systems and poses the serious health problems. The elements of greatest concern include Pb (Group 14), transition elements: Pd, Ni, Cr, Cu, Fe, Cd and Hg, and As (Group 15). Other different types of metals, like Cs (an alkali metal), Be, Sr, and Ba (alkaline earth metals), and U and Th (rare earth metals) also pose health problems [80]. Man's perturbation of nature's slowly occurring life cycle of metals includes (i) the extraction, smelting, and processing of metal bearing ores into products, (ii) the distribution and use of these products by industry and consumers, and (iii) the return of these metals in a concentrated form to the natural environment through disposal of processing wastes and the discard of spent products. The metal or metals thus become contaminants in the receiving environments. Part of the reason they become contaminants is seen within the description of the man-made life cycles above. They include (i) the rapidity of the man-made cycles relative to natural ones, (ii) the transfer of the metals from mines to random environmental locations where higher potentials of direct exposure occur, (iii) the relatively high concentrations of the metals in discarded products compared to those in the receiving environment, and (iv) the chemical form, or species, in which a metal is found in the receiving environmental system.

Environmental concerns drove an interest in metal species determination after the accidental mercurypoisoning in Minamata, Japan in the 1950s. Other environmentalsectors such as waste water treatment andwaste management will be reliant on metal speciesdeterminations. Environmental issues will be drivenwith the introduction of environmental regulations.These regulations may be set when commercial instrumentationis available and further communicationbetween ecotoxicologists, environmental and analyticalchemists is improved.Therefore,the concept of trace metal or elemental speciation has aroused increasing interest among the inorganic analytical chemistry community during the last 30 years [81]. Together with the progress in analytical instrumentation and analytical schemes, the need for species identification will bring new insights regarding the use and applicability of trace metal species determination. This knowledge is the result of the continuous evolution of approaches developed by environmental chemists, geochemists and biologically oriented chemists relying on solid chemical reasoning.

Environmental research has now clearly established that metals and metalloids occur in the environment as a large array of chemical species. They occur at low concentrations as free metal ions and are most commonly found in combined forms. For instance, these may be found as inorganic salts, oxyhydroxides, and charged or neutral organometallic entities. They can also be found integrated into larger biological molecular structures such as amino acids, proteins and metalloenzymes. All of these various chemical species undergo continuous chemical changes with kinetic and thermodynamic forces to constantly change the ecosystem. It has now been well established that the chemical species of a metal closely regulates its fundamental physico-chemical properties (liquid to solid and liquid to gas partitioning) and hence its mode and the occurrence of its bioaccumulation in the food chain. The type of species present also directly determines the toxicity of the metal in biota [82-88]. The toxicity of trace metals in environmental sciences is an area of increasing interest. In the environment, many diverse species of an element can be present, and different species of the same element can possess very different degrees of toxicity. Monitoring elemental species requires an analytical method that is sensitive and specific enough to resolve and quantify the individual species may constitute only a fraction of an element's total concentration in a sample.

The primary requirements for metal and metalloidspecies determination are related to the toxicityaspects regarding biological systems. This attitudetowards metal speciation is, however, changing andthe demand for speciation now comes from areasother than environmental analysis. Demands arenow increasing in industrial process control, thefood industry, and the biomedical and the pharmaceuticalfields. However, metal speciesdeterminations should be understood as a way toincrease and improve the quality of industrial processesat lower cost. Increasing informationexchange between academic research groups andtheir industrial partners will facilitate enhancedmetal speciation analyses. The nuclear industry should also benefit from thedevelopment of chemical species identification. Inthis field, the concept of determining chemical specieshas been developed. However, most of the analytical results are instrument-limited and rely on radiochemical procedures using gamma spectroscopic detection.

The rapid development of ultrasensitive techniquesusing coupled systems with ICP-MS detection shouldbring new insight into the field of chemical species determination of actinides. Table 1 lists different types of applications where chemical species determination is playing a fundamental role in the evolution of a particular subfield. Hundreds of mineral species contain iron as a constituent, and igneous rocks average about 5 percent iron content. The major iron ores are the minerals hematite (Fe_2O_3), magnetite (Fe_3O_4), limonite ($FeO(OH)\cdot nH_2O$), and siderite ($FeCO_3$). Iron is the most useful of all metals. It is also the cheapest available metal. Most is used to manufacture steel. Ordinary carbon steel is an alloy of iron with carbon (about 1.5%), with small amounts of other elements. Alloy steels are carbon steels with other additives such as nickel and chromium. Wrought iron is iron containing a very small amount of carbon, and is tough, malleable and less fusible than pure iron. Pig iron is an alloy containing about 3% carbon with varying amounts of sulfur, silicon, manganese and phosphorus. It is hard, brittle, fairly fusible and is used to produce other alloys including steel. Therefore, Iron is one the most important constituent of metallurgical industry. Hence determination of iron is very important. Zaijun et al. and Sarma et al. have analyzed Fe(III) by forming complex of Fe(III) with dimethyldithiocarbamate [89] and pyridoxal-4-phenyl-3-thiosemicarbazone [90], respectively. In sufficient amounts, copper salts can be poisonous to higher organisms as well. Copper is a naturally occurring element that is present in drinking water.Stagnation of water in pipes and plumbing fixtures containing copper andcopper alloys in distribution systems and household plumbing allows leachingand increases water copper levels. Characteristics of the water, including increased acidity, increased temperature, and reduced hardness, can increase theleaching of copper into the water. Acute ingestion of excess copper in drinking water is associated with adverse health effects, including acute

Table 1. Determination of metal species in the selected fields of development

Element	Selected field	Applications
Al	Industrial	Polymerisation products
	Biomedical	Chemical forms in serum and biological
	Food	fluids
		Chemical forms in foodstuffs
Sb	Environmental	Redox and organometallic species in sea
	Food	and soil
		Redox and organometals in foodstuffs
As	Environmental	Redox and organometallic species
	Biomedical	As in proteins, haemoglobin and
	Food	biological fluids
	Industrial hygiene	As in foodstuffs
		Workplace atmosphere
Cd	Environmental	Cd complexes and metalloproteins
Cr	Environmental	Redox species in the environment
	Food	Food stuffs
Cu	Environmental	Redox species in soil and water samples
	Food	Species in foodstuffs
Fe	Environmental	Species in soil and water samples
	Food	Species in foodstuffs
	Biomedical	Fe in blood, serum and proteins
Pb	Environmental	Species in soil and water samples
	Industrial hygiene	Species in the atmosphere
P	Industrial hygiene	Phosphine in workplace
Hg	Environmental	Species in soil and water samples
	Industrial hygiene	Species in workplace
	Food	Species in foodstuffs
Pt	Environmental	Species in soil and water samples
	Pharmaceutical	Cis-Pt formulations
	Biomedical	Cis-Pt in cancer therapy
Se	Environmental	Redox and organometallic species
	Food	Species in foodstuffs
Sn	Environmental	Species in soil and water samples
	Industrial	Formulations in catalysts
Th	Industrial	Species in effluents and waste waters
	Environmental	Species in soil, water and nuclear sites
U	Industrial	Species in effluents and waste waters
	Environmental	Species in soil, water and nuclear sites

gastrointestinal disturbances, and liver toxicity. A model for liver toxicity might be derivedfrom patients with Wilson disease, which causes abnormal copper regulatorymechanisms that result in accumulation of excess copper. Thecurrent EPA MCLG of 1.3 milligrams per liter (mg/L) for copper in drinkingwater is based on the need to protect against adverse gastrointestinal effects. Thus, the determination of Cu(II) is becoming important due to the ill effects of excess copper in drinking water and soil. Therefore, there is a need to develop novel methods for the determination of Cu(II) that might be important to know the exact concentration of Cu(II) in soil and water system. So far, researchers have developed methods for estimating Cu(II) by forming Cu(II) complexes with electron rich species such as 2-ethanolimino-2-pentylidino-4-one [91], 1-(2-quinolylazo)-2,4,5-trihydroxybenzene [92], 1,1,1-trifluoro-3-(2-thenoyl)acetone [93], diacetyl monooxime [94]. These metal-ligand complexes were monitored spectrophometrically and utilized to know the concentration of Cu(II). Uranium is a silvery-white metallic chemical element in the actinide series of the periodic table.The actinides refer to the fourteen elements with atomic numbers 90 through 103 i.e. from thorium to lawrencium through uranium (Atomic number 92). Uranium is radioactive, long-lived, and highly toxic element. Uranium-238, the most prevalent isotope in uranium ore, has a half-life of about 4.5 billion years. Uranium-238 decays by alpha emission into thorium-234, which itself decays by beta emission to protactinium-234, which decays by beta emission to uranium-234, and so on. The various decay products, (sometimes referred to as "progeny" or "daughters") form a series starting at uranium-238. After several more alpha and beta decays, the series ends with the stable isotope lead-206. As long as it remains outside the body, uranium poses little health hazard (mainly from the gamma-rays). If inhaled or ingested, however, its radioactivity poses increased risks of lung cancer and bone cancer. Uranium is also chemically toxic at high concentrations and can cause damage to internal organs, notably the kidneys. Uranium content of land waters in excess of 1 ppb (1μg L^{-1}) is regarded as anomaly.

Uranium occurs in +III, +IV, +V and +VI oxidation states. However compounds of U(VI) is of considerable importance to analytical-inorganic chemists. Uranium species of interest is the uranyl ion, UO_2^{2+} which is stable, highly soluble and mobile in aqueous phase. Uranyl ion can be found in soils and in low pH-water runoff in and around nuclear waste sites and processing facilities. The uranyl unit consists of a uranium centre with a formal charge of +6 coordinated to two double bonded oxygen atoms for a linear dioxo cation. This unit is highly stable and binds to other ligands via the formation of U-O bonds in a plane perpendicular to the axis of the uranyl ion.
The spectrophotometric methods are virtually the commonestmethods in chemical analysis. Colour reactions of practicalvalue have now been found for almost all inorganic ions.An increasing number of analytical methods based on metal-ligand interactions arebased on the corresponding colour reactions. Electron rich speciessuch as o-hydroxypropiophenone isonicotinoyl hydrazone [95], 2-hydroxybenzohydroxamic acid [96], 4-(2-pyridylazo)resorcinol [97], 2-(2-thiazolylazo)-p-cresol [98] were reacted with U(VI) to form coloured complexes and thus exploited for the determination of U(VI).

Thorium is a naturally occurring, slightly radioactivemetal. It is estimated to be about three to four times more abundant than uranium in the earth's crust. It has been considered a waste product in mining rare earths, so its abundance is high and cost low. Monazite is the primary source of thorium. Thorium is present in very small quantities in virtually all rock, soil, water, plants and animals. Wherehigh concentrations occur in rock, thorium may be mined and refined, producing waste products suchas mill tailings. If not properly controlled, wind and water can introduce the tailings into the wideenvironment[99,100]. If inhaled as a dust, some thorium may remain in the lungs for long periods oftime, depending on its chemical form. If ingested, thorium typically leaves the body through face andurine within several days. The small amount of thorium left in the body will enter the blood stream andbe deposited on the bones where it may remain for many years. Studies have shown that inhalingthorium dust causes an increased risk of developing lung cancer, and cancer of the pancreas. Bonescancer risk is also increased because thorium may be stored in bone[101-103]. Thorium occurs in +III and +IV oxidation states. Th(IV) oxidation state is the only state observed in aqueous solution [104]. This is untypical of the actinides where +III tends to be dominant state. The precipitation with oxalate from dilute acid solution is a principal means of separation from most elements including zirconium and titanium. Spectrophotometry enjoys a significant role in the determination of traces of metals due to simplicity and accuracy. The problem of chemical analysis generally follows two steps: (i) Separation of the desired constituent and (ii) determination of the desired analyte. Much research has been carried out for the determination Th(IV) by employing specific electron rich species such as 2-hydroxy-1-napthaldehyde isonicotinoylhydrazone [105], Semi-xylenol

orange [106], 2-carboxy maleianilic acid [107], N-(2-hydroxyethyl)ethylenediaminetriacetic acid [108] and four azo compounds based on I-phenyl-2, 3-dimethylpyrazoline-5-one nucleus namely 4-phenylazo- (2-hydroxy, 5-x) 1-pheny1-2, 3-dimethy1-pyrazoline-5-one, where x= H (1), OH (II), COOH (III) and NH_2 (IV) [109]. These Th(IV)-ligand complexes have given characterstic colour reactions. This fact of complex formation was exploited and thereby utilized for the determination of Th(IV) by spectrophotometric method.

Sampling

There is no general agreement on the solutions preferred for the various components in sediment or soils to be extracted, due mostly to the matrix effect involved in the heterogeneous chemical processes [110]. All factors have to be critically considered when an extractant for a specific investigation is chosen. Important factors are the aim of the study, the type of solid materials, and the elements of interest. Partial dissolution techniques should include reagents that are sensitive to only one of the various components significant in trace metal binding. Whatever extraction procedure is selected, the validity of selective extraction results primarily depends on the sample collection and preservation prior to analysis. A sampling plan has to be established prior to sampling. The purpose and expectation of a sampling program must be realistic and can never surpass the measurement and sample limitations. Moreover,costs and benefits must be considered in the design of every measurement program.The total variance of an analysis (s^2_{total}) is expressed as:

$$s^2_{total} = s^2_{measurement} + s^2_{sampling}$$

where $s^2_{measurement}$ and $s^2_{sampling}$ are the variances due to the measurement and sampling, respectively [111]. The measurement, sampling plans and operations must be designed and accomplished so that the individual components may be evaluated. Sampling uncertainty may contain systematic and random components arising from the sampling procedure. In environmental sampling, the act of sample removal from its natural environment can disturb stable or meta-stable equilibria. If the test portion is not representative of the original material, it will not be possible to relate the analytical result to the originalmaterial, no matter how good the analytical method is nor how carefully the analysis is performed.Further, sampling errors cannot be controlled by the use of standards or reference materials.

Sampling of sediments and soils

Because of the heterogeneity and complex nature of sediments, care should be taken during samplingand analysis to minimize changes in speciation due to changes in the environmental conditions of thesystem. Sampling for pollution mapping has to consider the heterogeneity of the deposit by methodssuch as particle size analysis and geochemical normalization. Sediment sampling must avoid alterationof natural biogeochemical processes, which would affect results by the unrepresentativeness of the originalequilibrium. Consequently, sampling variance and artifacts introduced during processing of samplescan be more than an order of magnitude greater than analytical measurement variances in trace elementspeciation [112]. Schoer [113] has studied the effect of particle size of sediments on the adsorption capacity. Variations in the behavior of different elements with particle size is attributed largely to differences intheir relative potential for sorption on clay minerals, hydrous oxides, and organic matter surfaces, all ofwhich tend to be concentrated in smaller grain sizes. The maximum concentration of organic carbon inthe sediment samples was found in a size range of 2–6.3 μm, whereas smaller fractions showed onlytraces of organic carbon. On the other hand, easily reducible manganese reached its highest concentrationin the fraction of <2 μm. Appropriate comparability among oxide sediment samples collected atdifferent times and places from a given aquatic system and between different systems can be obtainedmost easily by analyzing the fine-grained fraction of sediment.Some investigations have also pointed to a relation between specific surface, grain size fraction,and the speciation of trace elements in sediments. Amorphous Fe-oxide precipitates appear to be mostsignificant in affecting both surface area and sediment trace metal levels. It was found that external surfacearea, determined by Brunauer–Emmett–Teller (BET) method, is a function of both grain size andof composition of geochemical phase [114]. Suspended particulate matter sampling is mainly carried outby filtration. Such samples are of limited utility for studies of the speciation of elements in solids. Inrecent years, suspended sediment recovery by continuous-flow centrifugation has commonly been usedto obtain sufficient sample for speciation, up to a few grams to carry out all the analysis: particle sizedistribution, mineralogy, total and sequential extractions content. Etcheber et al. [115] provided a comparativestudy of suspended particle matter separation by filtration, continuous-flow centrifugation, andshallow water sediment traps. Although particles were separated by density, rather than size, the continuous-flow centrifugation technique was preferred due to its speed and high recovery rate. The continuous-flow separation technique is simpler to use especially on the open sea, where suspended sedimentconcentrations are low. Trace elements in suspended particulate matter from open North Sea havebeen measured for particle size distribution, specific surface, bulk concentration, and partitioning betweenfive sequential extraction fractions [116].

The trace element concentration in soils and sediments are normally much higher than those for water samples, many precautionary steps taken relating to sample container preparations and sampling of waters are equally applicable to soil and sediments. Soil composition may vary greatly over a small area. Samples have to be taken from a number of locations to obtain a suitable average composition studies, the source of contamination and its mobility within the soil should be taken into account. Often pollutants deposited from the atmosphere are immobile and will remain within the surface layer. If the soil is disturbed, sample should be taken from the whole of the disturbed area. For landfill sites, samples should be taken over the complete depth of the land fill. The samples collected should be representative of the sampled land. In many cases it is important to take sub samples within a defined area in order to provide a representative composite sample. Sediment sampling is normally carried out using a tube corer or a grab sampler. Core samplers are used for shallow areas. The tube is immersed with valve system open. The valve is then closed to permit the sample to be withdrawn. Just before breaking the surface of the water, the tube is sealed to preserve the sediment structure so that sections corresponding to different depths in the sediments can be analyzed. Grab

samples are used where the sediment is loose so that there is no vertical structure.

Sample preparation

Soil and sediment samples should be stored in sealed polythene containers at 40^0C until arrival at the laboratory. The samples are seperated into particle sizes by wet or dry sieving using 63µm or 20µm nylon sieves. Moist sediments are often used for metal specification analysis. Such samples should be sealed under the nitrogen in polyethylene containers and frozen. Before chemical analysis, the samples should be homogenised and dried (30-60^0C) to a constant weight. Homogenisation is achieved by a grinding mill or agate pestle and mortar. The powdered sample is poured into a cone shaped heap, divided into four equal parts. Two opposite quarters are combined and reconed. The process is repeated until the amount of the sample is reduced to that required for analysis.

Analytical methodology

Total metalsdetermination requires the extraction of the elementfrom a complex matrix. The detection step usingatomic absorption spectroscopic techniques is usually performed without matrix removal, which may lead to severeinterference problems. Most of the analytical instrumental development has been associated with the spectroscopic reduction of these interferences in order to yield improved quality data. Despite the important instrumental progress, interlaboratory comparison exercises show major discrepancies in the results obtained from the laboratories involved. Metal species determination requires careful extraction and preservation ofthe analytes. These steps considerably minimise thefinal amount of material introduced at the detectionstage and hence considerably reduce spectroscopic interferences compared with total metals determination.European Economic Communityinterlaboratory exercises have frequentlyshown that a better agreement is obtained by participantsperforming species determination comparedwith those measuring the total metals content for the certification of reference materials.Part of the future development in this area will beclosely related to the introduction of commercial instrumentation for speciation. Many chemical approaches can be solved with simple low-cost instrumentation. There is, however, a concern with manymethods being developed that use expensive massspectrometric detection systems only available in certain laboratories.

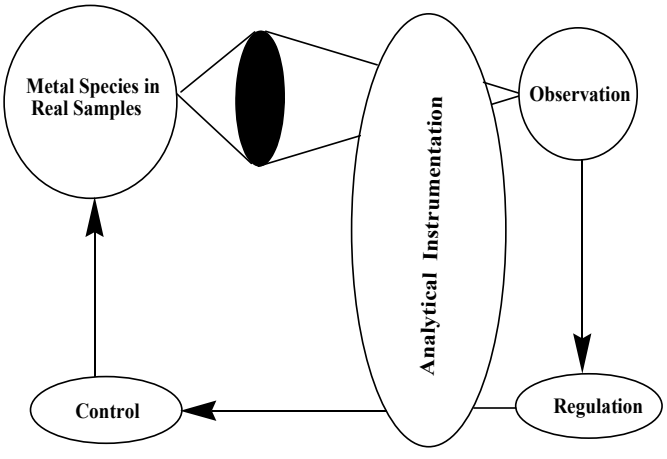


Fig. 1. Importance of analytical instrumentation in speciation of metals

Fig. 1 illustrates the importance of analyticalinstrumentation in environmental decisionmakingprocesses. Biological systems can be responded tochemical species and the species integrity can be maintained using appropriate instrumentation.Regulation and control are correcttools so that decision making may be rationalised.Applications of metal and metalloid speciesdeterminations are numerous and speciation analysesshould soon be substituted for current total metaldeterminations.Many areas, however, cannot waitfor the implementationof regulations. The food, pharmaceutical,biomedical and chemical industries willrapidlyrequire high-quality information obtained by metalspecies determination to improve and consolidatetheir developments. Many organic compounds (natural and synthetic) possess electron rich centres due to the presence of N,O and S are capable of forming metal complexes. Metal complexes have spectacular colors. These colors arise from the absorption of lightwhich excites electrons within the metal's orbitals or, in the case of charge-transfer, excites electrons from metal-based orbitals to ligand-based orbitals (or the reverse). The interaction of metal ions with electron rich species leading to the formation of metal complexes is exploited to develop analytical procedures for the estimation of metal ions in soil, sea, sewage and natural waters. This aspect of study is being helpful in monitoring the pollution level. Instrumental analysis is opened many doors to scientific progress in support of both traditional core areas of chemistry, and new ones like biotechnology, material chemistry, environmental chemistry, chemical toxicology and small domain chemistry. UV-visible spectrophotometric methods continue to be popular for carrying out single component assays on a variety of metal ions via complex formation with suitable ligands. Represantative examples of UV-visible spectrophotometric methods of metal analysis [117-137] that have been published are given in Table 2.

Validation

The method to be fit for the determination of metal ions in soil and natural water samples, the method must meet the following validation characteristics. Typical validation characteristics (Table 3), which should be considered are as follows:

- Selectivity/ Specificity
- Linearity
- Range
- Accuracy
- Precision
- Limits of detection and quantitation
- Robustness / ruggedness

Selectivity/specificity

Selectivty of a method refers to the extent to which it can determine particular analyte(s) in a complex mixture without interference from other components in the mixture. The terms selectivity and specificity have been used interchangeably. The term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method, which provides responses for a number of chemical entities that may or may not bedistinguished from each other. If the response is distinguished from all other responses the method is said to be selective. Since there are very few methods that respond to only one analyte, the term selectivity is usually more appropriate than specificity.

Table 2. UV-visible spectrophotometric methods for the determination of metal ion in environmental samples

Metal ions	Reagents used	λ_{max} (nm)	References
Cu(II)	Chlor(phenyl) glyoxime	290.5	[117]
U(VI)	Meloxicam	398	[118]
U(VI)	Piroxicam	390	[119]
Fe(III)	Piroxicam	495	[120]
Cu(II)	Cefixime	336	[121]
Ni(II)	2-Hydroxy-3-methoxybenzaldehyde thiosemicarbazone	410	[122]
Bi(III)	1-amino-4,4,6-trimethyl(1H,4H) pyrimidine 2-thiol	470	[123]
Pb(II)	Benzoic acid azo phenylcalix[4]arene	440	[124]
Tl(III)	4-(4′-*N*,*N*-dimethylaminophenyl)urazole	514	[125]
As(III)	2-(5-bromo-2-pyridylazo)-5-di-ethylaminophenol	560	[126]
Ni(II)	Dibromo-*p*-methyl-carboxyazo	625	[127]
Hg(II)	Diphenylthiocarbazone	488	[128]
Fe(II)	2,2'-pyridine	522	[129]
V(V)	Variamine blue	570	[130]
Pd(II)	2-(2-quinolylazo)-5-diethylaminobenzoic acid	628	[131]
Cr(VI) and V(V)	3,4-Dihydroxybenzaldehyde isonicotinoyl hydrazone	400 & 360	[132]
U(VI)	ArsenazoIII	651	[133]
La(III)	1-(-2-pyridylazo)-2-naphthol	530	[134]
Au(II)	2-carboxyl-1-naphthalthiorhodanine	540	[135]
Pb(II)	Chromazurol S	520	[136]
Cr(VI)	Ferroin	510	[137]

Table 3. Validation characteristics normally evaluated for different types of test procedure and the minimum number of determinations required (if applicable)

Validation characteristics	Minimum number	Test procedure			
		Identity	Impurities		Assay
			Quantitative	Limit	
Specificity	-	Yes	Yes	Yes	Yes
Linearity	5 concentrations	No	Yes	No	Yes
Range	-	No	Yes	No	Yes
Accuracy	9 determinations over 3 concentration levels (e.g. 3 × 3)	No	Yes	No	Yes
Precision					
Repeatability	6 determinations at 100% or 9 determinations over 3concentration levels (e.g. 3 × 3)	No	Yes	No	Yes
Intermediate Precision/ rep- roducibility	2-series	No	Yes	No	Yes
Detection limit	-	No	No	Yes	No
Quantitation limit	-	No	Yes	No	No

The International Union of Pure and Applied Chemistry (IUPAC) has expressed the view that "Specificity is the ultimate of selectivity". The IUPAC discourages the use of the term specificity and encourages the use of the term selectivity.

Linearity

The linearity is the ability of the method to produce test results which are proportional to the concentration (amount) of analyte in samples within a given concentration range, either directly or by means of a well-defined mathematical transformation. Linearity should be determined by using a minimum of five standards whose concentration span 80-120% of the expected concentration range. The linearity of a method should be established by the inspection of the plot of the instrumental response versus the initial concentration of analyte. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of the regression line using least square method.

Range

The specified range is derived from the linearity studies. The range of the proposed procedure is the interval between the upper and lower concentration (amount) of analyte in the sample for which it has been demonstrated that the analytical method has suitable levels of precision, accuracy and linearity.

Accuracy

The accuracy of a method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value. Accuracy may be measured in different ways and the method should be appropriate to the matrix. The accuracy of an analytical method may be determined by any of the following ways:

- Analyzing a sample of known concentration and comparing the measured value to the 'true' value. However, a well characterized sample (e.g. reference standard) must be used.
- Standard addition method. In the standard addition method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed. The difference between the results of the two assays is compared with the expected answer.

Precision

According to International Conference on Harmonisation (ICH), the precision is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions and may be considered at three levels:

- repeatability
- intermediate precision
- reproducibility

Repeatability: It is the precision obtained by independent test results with the same method on identical test material in the same laboratory by the same operator using the same equipment within short interval of time. It is also termed as intra-assay precision [138]. Sometimes it is also termed as within run or within day precision.

- **Intermediate precision:** It expresses within-laboratories variations: different days, different analysts, different equipment etc. The International Organization for Standardization (ISO) definitions used the term "M-factor different intermediate precision" where the M-factor expresses the number of factors (reference standard, operator, equipment, laboratory or time) that differ between successive determinations [139]. Intermediate precision is sometimes also called between-run, between-day or inter-assay precision.
- **Reproducibility**: It is the precision obtained within the same method on identical test material in different laboratories with different operators using different equipments [140].

Limits of detection and quantitation: Limit of detection (LOD) determines the lowest amount of analyte that can be detected, as it (the analyte) yields instrumental response greater than a blank, but cannot be quantified. It is a parameter of "limit test" and expected to produce a response, which is significantly different from that of a blank. On the other hand limit of quantitation (LOQ) is a parameter of "determination test" and can be defined as the lowest concentration of the analyte that can be measured and quantified with acceptable precision and accuracy. The most common definition of LOD and LOQ is the analyte concentration for which the signal exceeds that for a realistic analytical blank by three and ten times of the standard deviation, respectively. Several approaches have been given in the ICH guidelines to determine the detection and quantitation limits (Table 4).

Robustness/ruggedness: The "robustness / ruggedness" of an analytical procedure is defined [141] as a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters and provides an indication of its reliability during normal usage. Ruggedness is a measure for the susceptibility of a method to small changes that might occur during routine analysis like small changes of pH values,mobile phase composition, temperature etc. Full validation must not necessarily include ruggedness testing; it can, however, be very helpful during the method development / prevalidation phase, as problems that may occur during validation areoften detected in advance. Ruggedness should be tested, if a method is supposed to be transferred to another laboratory.

Table 4. Approaches for determining limits of detection and quantization

Approach	Detection limit	Quantitation limit
Visual evolution	Minimum level detection	Minimum level quantifiable
Signal-to-noise	3:1or 2:1	10:1
Standard deviation of the response (S_o)[a] and the slope(b)	3.3 × (S_o)/b	10.0 × (S_o)/b

[a]Standard deviation of the blank, residual standard deviation of the calibration line, or standard deviation of the intercept.

Statistical Analysis

Recent trend in the determination of metal emphasizes the use of statistical analysis for evaluation of the method performance which includes the following parameters [142,143]:

- Mean
- Standard deviation, variance & standard analytical error
- Relative standard deviation
- Coefficient of correlation
- Regression line
- Variance
- Errors in the slope and the intercept
- Confidence limit for the slope and the intercept
- Error in the concentration
- Equivalence testing
- Interval hypothesis

Mean. It is the sum of all the measurements divided by the number of measurements. It is calculated by the following expression:

$$\bar{x} = \sum_i x_i / \mathrm{n}$$

Standard deviation, variance &standard analytical error. The most useful measure of spread is the standard deviation, S.D. This is defined by the formula:

$$S.D. = \sqrt{\sum_i (x_i - \bar{x})^2/(\mathrm{n}-1)}$$

The square of S.D. is a very important quantity known as the variance which is useful in propagation of error. The standard analytical error (SAE) of the mean is calculated by SAE = S.D. / √n

Relative standard deviation

It is calculated by

$$RSD = {}^{SD}/_{\bar{x}} \times 100$$

The RSD (also called coefficient of variation), the units of which are percent is an example of relative error.

Coefficient of correlation

When using instrumental methods, it is necessary to carry out a calibration process by using a series of samples (standard) each having a known concentration of analyte. Two statistical procedures should be applied to the calibration curve:

- Test whether the graph is linear or in the form of a curve
- Find the best straight line (or curve) through the data points

Linearity is judged by correlation coefficient, 'r', which can be calculated for a calibration curve to ascertain the degree of correlation between the measured instrumental variable and the sample concentration.

$$r = \frac{n\Sigma x_i y_i - \Sigma x_i \Sigma y_i}{\sqrt{[n\Sigma x_i^2 - (\Sigma x_i)^2][n\Sigma y_i^2 - (\Sigma y_i)^2]}}$$

Where n = number of data points

The maximum value of r is 1. When this occurs there is exact correlation between the two variables (x and y). When the value of r is zero (xy = 0), there is complete independence of the variables. The minimum value of r is -1, indicates that the assumed dependence is opposite to what exists (Fig. 2). As a general rule, 0.90 <r< 0.95 indicates a fair curve, 0.95 < r < 0.99 as a good curve, and r > 0.99 includes excellent linearity.

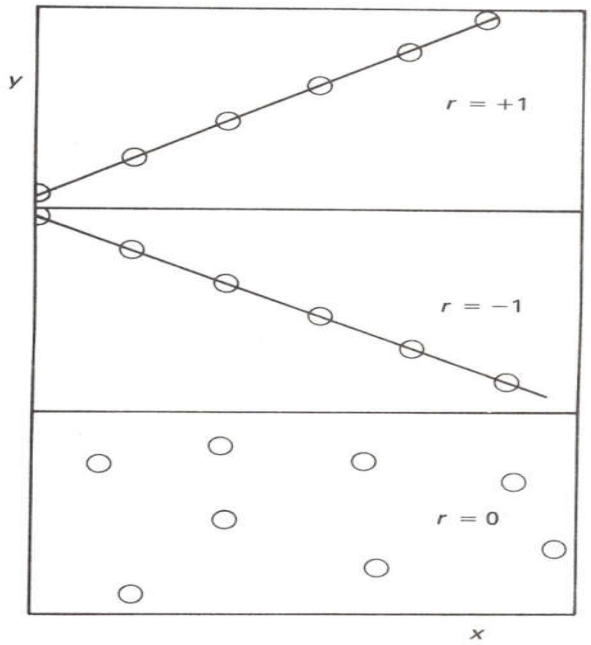


Fig. 2. Linearity with the values of correlation coefficient

Regression line

The best straight line through a series of experimental points is that line for which the sum of the squares of the deviation of the points from the line is minimum. Besides, determining a straight line, uncertainties in the use of calibration graph for analysis of unknown samples can be specified by this method of least squares. The equation of the straight line is

A = a + b C

Where A = instrumental response (i.e. absorbance), b = slope, a = intercept, C = concentration of the standards.

To obtain the regression line A on C, the slope 'b' of the line and the intercept 'a' on the y-axis are given by the following equations:

$$b = \frac{\Sigma x_i y_i - [(\Sigma x_i \Sigma y_i)/n]}{\Sigma x_i^2 - [(\Sigma x_i)^2/n]}$$

$$a = \bar{y} - b\bar{x}$$

Where $\bar{x}$ = mean of all the values of x_i and $\bar{y}$ = mean of all the values of y_i.

Errors in the slope and the intercept

The determination of errors in the slope (b) and intercept (a) of the regression line may be calculated by first calculating S_0(standard deviation of the calibration line) from the following equation:

$$S_o = \sqrt{\Sigma(y_i - \bar{y})^2/(n-2)}$$

where $\bar{y}$ values are obtained from calculated regression line for given values of x; once the value S_o has been obtained, both the standard deviations of the slope S_b and the intercept S_a can be obtained from the following equations

$$S_b = S_o\sqrt{\Sigma(x_i - \bar{x})^2}$$

$$S_a = S_o\sqrt{\Sigma x_i^2/n\Sigma(x_i - \bar{x})^2}$$

Confidence limit for the slope and the intercept

It determines whether the slope and/or intercept of a line differ significantly from a particular or predicted value. It can be calculated [144] in the following manner:

- b $\pm t$ S_b(for slope)
- a $\pm t$ S_a(for intercept)

where t = tabulated 't' value at desired confidence level for (n-2) degrees of freedom.

Error in the concentration

The determination of the erro

$$S_c = \frac{S_0}{b}\left[1 + \frac{1}{n} + \frac{(y-\bar{y})^2}{b^2\,\Sigma(x-\bar{x})^2}\right]^{1/2}$$

where $\bar{x}$ and $\bar{y}$ are the average concentration and absorbance values, respectively, for 'n' standard solutions.

Equivalence testing

An important property of an analytical method is that it should be free from the systematic error (bias). Determining bias involves analyzing one or more standard reference materials whose analyte concentration is known. However, random errors make it unlikely that, the measured amount will equal to the known amount even when no systematic errors are present. In order to decide whether the difference between the observed and standard values can be accounted for by random variation, a statistical test i.e. a significance test is used for the interpretation of analytical data.

- **Student's t-test:** Here comparison is made between two sets of replicate measurements made by two different methods; one is the test method while other is accepted (reference method).

$$\pm t = \frac{\overline{x_1} - \overline{x_2}}{S_p}\sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

where

$\overline{x_1}$ = mean from the test method

$\overline{x_2}$ = mean from the accepted (reference) method

$n_1$and n_2 = number of measurements
S_p = pooled standard deviation of the individual measurements of two sets is given by

$$S_p = \sqrt{\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2}}$$

A statistical *t*-value is calculated and compared with a tabulated value for the given number of tests at the desired confidence level. If $t_{cal} > t_{tab}$ then there is significant difference between the results obtained by the two methods at the given confidence level, but if $t_{cal} < t_{tab}$ then there is no significant difference between the methods. It is an accuracy-indicating test.

- **F-Test:** This test indicates whether there is a significant difference between the two methods (i.e. the new method and the accepted reference method). It can be represented as:

$$F = \frac{S_1^2}{S_2^2}$$

where $S_1^2 > S_2^2$

If $F_{cal} > F_{tab}$ at the selected confidence level, then there is a significant difference between the variances of the two methods.

Interval hypothesis

For a method, a bias of ± 2.0 % is acceptable [145] and can be calculated statistically [146] using the following quadratic equation:

$$\theta^2\left(\overline{x_1^2} - S_p^2 {t_{tab}}^2/n_1\right) + \theta(-2\overline{x_1}\,\overline{x_2}) + \left(\overline{x_2^2} - S_p^2 {t_{tab}}^2/n_2\right) = 0$$

where $\overline{x}_1$ and $\overline{x}_2$ are the means of methods 1 and 2, based on n_1 and n_2 measurements, respectively. S_p is the pooled standard deviation and t_{tab} is the tabulated one sided *t*-value, with $n_1 + n_2 - 2$ degrees of freedom at the specified level of significance.

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