Chapter (1) Introduction

1.1. Chromium occurrence in the environment

Chromium occurs in the environment in various chemically, physically and morphologically different forms. The element is never found free in nature due to its chemical reactivity. It is the 21st most abundant element in the Earth’s crust with an average concentration of 100 ppm [1]. It is found in nature in the chromite $FeO.Cr_2O_3$ and crocoite $PbCrO_4$ minerals. Many rocks contain large quantities of chromium. On the other hand, water contains low levels of chromium. The maximum concentration of chromium (VI) permitted in potable water is 5 µg/L [2]. Most surface water contains very low level of chromium, except the wastewater coming from industries. Industries dealing with paints, pigments, dyes, textile, leather, etc. are an important source of discharge of chromium in the environment.

Many minerals contain chromium as a major element and others contain tens of percent chromium. However, only chromite mineral occurs in large enough quantities to be a commercial source of chromium. Chromite can be found in many different rock types, but the host rocks for economically important chromite deposits are peridotite and norite. These are distinctive rocks composed mainly of the minerals olivine and pyroxene (peridotite) and pyroxene and plagioclase (norite). These rocks occur primarily in two geologic settings: layered intrusions, which are large bodies of layered igneous rock that cooled very slowly in large underground chambers of molten rock; and ophiolites, which are large pieces of the oceanic crust and mantle that have been thrust over continental rocks by the same tectonic forces that cause continental drift [3].

Because chromite deposits in layered intrusions tend to be tabular in form they are known asstratiform deposits, whereas those in ophiolites are typically podlike or irregular in form, are known podiform deposits. Other sources of chromite are beach sands derived from chromite-containing rocks and laterites that are weathering products of peridotite. Laterites are more widely known as sources of nickel and cobalt. Beach sands and laterites historically have been a minor source of chromite [3].

1.2. Physical and chemical properties for chromium

Chromium is a silver – grey with a relative atomic mass of 51.996, an atomic number of 24, melting point of 1875C°, boiling point of 2642C° and density of 7.190 kg/dm^3. It is belong to group VI of the periodic table. It has a body centered, cubic crystal structure. It passivated by oxygen forming a thin protective oxide surface layer which prevent oxidation of the underlying metal. It doesn’t tarnish in air when heated, it burns and forms the green chromic oxide [4].

Chromium is one of the so-called transition elements, meaning that it has valence electrons in two shells instead of one. Chromium exhibits a wide range of possible oxidation states, where the +3 state is most stable energetically; the +3 and +6 states are most commonly observed in chromium compounds, whereas the +1, +4 and +5 states are rare [5]. The electron shell configuration of chromium is 2, 8, 13, and 1 and the filling orbital is $3d^5$. It is soluble in H_2SO_4, HCl, HNO_3, and aqua regia. It resists corrosion and oxidation. When used in steel at greater that 10 wt% it forms a stable oxide surface layer, which makes it particularly useful in making stainless steel and other specialty steels to ward off the corrosive effects of water. The ability of chromium to resist corrosion and accept a high polish.

Air and water have no action on chromium at ordinary temperature but heating in air gives Cr_2O_3. It reacts readily with HCl, H_2SO_4 and $HClO_4$ but is passive toward HNO_3, due to the formation of protective layer and resists oxidation in air, chromium has 4 isotopes of masses 50, 52, 53 and 54 [5].

Chromium can exist in several chemical forms displaying oxidation numbers from (0 to VI). Only two of them, Cr (Ш) and Cr (VI) are stable enough to occur in the environment [6, 7].

These two oxidation states of chromium present in the environment are drastically different in charge, physicochemical properties as well as chemical and biological activities.

In the hexavalent state, chromium exists as oxo species such as (CrO_3) and $(CrO_4)^{2-}$ that are strongly oxidizing. In solution, hexvalent chromium exists as hydrochromate ($HCrO_4$), chromate $(CrO_4)^{2-}$ and dichromate $(Cr_2O_7)^{2-}$ ionic species. The proportion of each ion in solution is pH dependent. In basic and neutral pH, the chromate form predominates. As the pH is lowered, the hydrochromate concentration increases. At very low pH, the dichromate species predominate [6, 8].

1.3. Common uses of chromium

Chromium has a wide range of uses in chemicals, and refractories. It is one of the nation’s most important strategic materials. The most important application of Cr in the metallurgic industry is its use as an alloying element in steels. The two most important function of chromium in steels are improving the mechanical properties particularly harden ability and increasing the corrosion resistance [9]. It is an important engineering alloy used throughout industry in machinery, containers and pipes. Chromium is also used in chemicals for a variety of purposes. In tanning leather industries, chromium compounds are used in the electroplating industry as both an additive and as a highly powerful oxidizing agent. While there are some direct uses of chromite ores, mostly known for the production of refractory brick, the vast majority of ore is either oxidized or reduced.

Chromium and its salts are used in the leather tanning industry, the manufacture of catalysts, pigments and paints, fungicides, the ceramic and glass industry, and in photography, and for chrome alloy and chromium metal production, chrome plating, and corrosion control [10, 11]

Chromite, the mineral from which chromium is extracted for use in the metallurgical and chemical industries, is used directly by the refractory industry to produce heat, spalling, corrosion, and abrasion-resistant bricks for metallurgical and high- temperature industrial mineral processing applications. Chromite is not mined domestically; thus, the United States is 100% dependent on imports to meet domestic chromites demand. Domestic chromium demand is met by import of chromite ore; chromium ferroalloys, chromium metal, or chromite ore; and chemicals; and by recycling [10, 11].

1.4. Environmental and health effects of chromium

Chromium is the recently recognized biologically essential trace metal. The first conclusive evidence demonstrating a metabolic role of chromium was obtained by Mertz and Schwarz in a series of investigation of which the first report appeared in 1955 [12, 13]**.**

Chromium is unique among regulated toxic elements in the environment in that different species of chromium. Specifically, Cr (Ш) and Cr (VI) are regulated in different ways based on their differing toxicities. The determination of micro amounts of chromium in soil and other naturally occurring materials are of considerable interest because of the contrasting biological effects of its two common oxidation states, chromium (III) and chromium (VI). Chromium (III) is an essential nutrient for maintaining normal physiological function [14] whereas, chromium (VI) is toxic [15]. It is difficult, however, to determine chromium directly in natural water samples because of its very low concentration level. It is known that an increase in the content in soils makes them infertile and toxic effect depends to some extent on the chromium oxidation state. On the other hand the introduction of chromium salts in to soil has some positive effects due to activation of some biochemical processes [16]. In save limit, Cr (Ш) is considered to be a trace element essential for the proper functioning of living organisms. It was responsible for the control of glucose and lipid metabolism in mammals. On the other hand, Cr (VI) exerts toxic effects on biological systems and it was found that the occupational exposure to hexavalent chromium leads to a variety of clinical problems [17]. Inhalation and retention of Cr (VI) – containing material can cause proforation of the nasal septum, asthma, bronchitis, inflammation of the larynx and liver and increased incidence of broncognic carcinoma [18]. Hexavalent chromium compounds are both skin and pulmonary sensitizers, producing a generalized irritation of the conjunctiva and mucous membranes, nasal perforations [19] and a contact dermatitis [20].

It can act directly at the site of contact or be absorbed into, or through, human tissue a very important difference is that chromate into passes through cellular membranes many orders of magnitude faster than do chromium species [20].

Chromium absorbed through the lungs into the blood system is excreted by the kidneys and the liver. The kidney appears to absorb chromium from the blood through the venal cortex and releases it into the urine. Thus, sampling of urine for chromium can be used for biological monitoring for certain types of welding fumes that contain water-soluble Cr (VI) [20].

1.5. Analytical techniques for chromium determination

A variety of approaches was reported to determine chromium in some environmental samples, minerals and rocks. These include spectrophotometry [21], high performance liquid chromatography [22], capillary electrophoresis [23], flow injection combined with mass spectrometry [24], inductively coupled plasma atomic emission [25, 26], flame and furnace atomic absorption spectroscopy [27-29], and mass spectrometry [30]. Detection limits for atomic absorption spectroscopy are in the range 0.05–0.2 g/litre [31].

1.5.1. Atomic absorption technique

The phenomenon of the absorption of radiation by atoms has been used for investigations in physics since the first part of the nineteenth century. The first analytical application of atomic absorption was to the determination of mercury by Muller. Not until 1955, when Walsh discovered the general usefulness of the approach to elemental analysis, was real analytical atomic absorption spectroscopy born. In the relatively short period of two and one decades since this development, atomic absorption spectroscopy has become one of the most important techniques for analysis of the element [32, 33].

1.5.1.1. Atomic absorption analysis

Atomic absorption technique [32, 33] is one of the most extensively used spectroscopic methods for determination of chromium. A major advantage of atomic absorption spectroscopy is the minimal interferences that occur from other elements in the sample as the hollow cathode lamp used is metal specific. Atomic absorption technique has also found wide application due to its simplicity and relatively low cost. For routine work, atomic absorption technique is more favorable in terms of precision and economy. Most samples require ashing and oxidation. In many systems, it is possible to analyze for chromium without extraction into an organic solvent.

The basic component of an atomic absorption spectrometer:

- Radiation source
- Atom cell (atomization)
- Device for isolation of atomic spectral lines (monochromator)
- Detector for UV- visible radiation
- Signal processor

New developments in atomic absorption technique are occurring rapidly, including improvements in design and new accessory modules for increasing sensitivity and reducing interferences. Among these are the deuterium background corrector for reducing interferences from light scattering in the flame due to the presence of extraneous cations, such as sodium and potassium, and the “ heated- graphite atomizer” for atomic absorption. The latter has great potential, especially for the analysis of chromium, but much exploratory work needs to be done before it becomes available for the routine analysis of chromium [32, 33].

There is almost no published information on the application of this method to the analysis of chromium, although private communications indicate extreme improvements in detection limits variable, which must be carefully controlled. It also appears that the viscosity of the sample contributes to the geometric distribution of the sample within the graphite tube, which affects the observed peak height. At this stage, it would be premature to suggest the use of this device until additional studies are conducted on some obvious variables that affect the analysis of chromium [34].

The popularity of this technique has increased in recent years due to decreased detection limits [34]. In flame atomic absorption spectrometry, either an air/acetylene or a nitrous oxide/acetylene flame is used to evaporate the solution and dissociate the sample into its component atoms. The flame atomic absorption technique uses an air acetylene flame to reach temperatures usually reported around 2400C° which is used to produce a good atom cell across which the absorption can be measured. Atomic absorption technique allows us to quantify the amount of a specific element present in a sample. However, some sample preparation may be required. Typically, this involves getting a solid sample into an acidified solution. The diluted solution is aspirated into a flame to atomize the sample. An element-specific or resonant wavelength of light passes through the atomized sample cloud. The amount of light that is absorbed by the sample is directly proportional to the concentration of the ground state element of interest in that aspirated sample.

The use of a flame limits the excitation temperature reached by a sample to a maximum of approximately 2800C° with the (N_2O/acetylene flame). For many elements this is not a problem. Compounds of of the alkali metals, for example and many of the heavy metals are all atomized with good efficiency with either flame type, with typical flame atomic absorption detection limits in the sub-ppm range. However, there are a number of refractory elements which do not perform well with a flame source. This is because the maximum temperature reached, even with the N_2O/acetylene flame, is insufficient to break down compounds of these elements. As a result, flame atomic absorption sensitivity for these elements is not as good as other elemental analysis techniques [34].

1.5.1.2. Basis of atomic absorption technique

Atomic absorption is a physical process [35] involving the absorption by the free atoms of an element, of light at a wavelength specific to that element. If we consider a sample or sample solution burned in a flame or heated in a tube, the individual atoms of the sample are released to form a cloud inside the flame or tube. The energy levels are labeled from E_0 the ground state; to $E_{\infty,}$ at which stage the electron has enough energy to break away from the atom, i.e. ionization occurs. For an unexcited atom, each electron is in the ground state. To excite the atom, one or more electron can be raised from the ground energy level to the first or higher energy levels by the absorption of energy by the atom. This energy can be supplied by photons. The photon energy equal to the difference between the two levels E_0

$_{\rightarrow 1}$ =hv. The wavelength of such photon =hc/ $E_{0\ \rightarrow\ 1}$ this wavelength is called the resonance wavelength. For atomic absorption instrument purposes, therefore, we can use an emission spectrum of the analyte element, generated by a hollow cathode lamp or electrode discharge lamp. Whereas, light from a hollow cathode lamp (selected based on the element to be analyzed) passes through the cloud of atoms, the atoms of interest absorb the light from the lamp. This is measured by a detector, and used to calculate the concentration of that element in the original sample.

1.5.1.3. Atomic absorption interference

Non analytical spectroscopists are often unfamiliar with interference problem in atomic absorption spectroscopy. The vast majority of elements have interference problems that are complex and not always predictable. This has very serious consequences. Atomic absorption has only available as analytical tool since 1955. During the first eight years few practical applications were published. Only since 1964 has there been a proliferation analytical application investigated. For this reason it is important to stress that quantitative information about interferences in many cases may still be locking. [33 and 36]**.**

1.5.1.3.1. Spectral interference

This type of interference occurs when the radiation being measured is attenuated by substances other than the atoms of interest [37]. Atomic spectral interference occurs when the absorption profile of another element overlaps that of the analyte within the spectral line width of the emission line of the source. Because of the narrow width of atomic emission lines, atomic spectral interference causes very few problems advantage compared to flames.

Molecular absorption occurs when a molecular species in the atomizer has absorption occurs when a molecular species in the atomizer has an absorption profile that overlaps that of the element of interest. This problem is very serious in the wavelength below 2500A°. Molecular absorption profiles are relatively broad compared to atomic absorption profiles and usually have narrow rotational lines superimposed on this continuum. If one of the latter coincides with the atomic absorption line, light scattering occurs [37].

Atomic absorption is very specific for a given element; the wavelength is so remote that the technique is generally recognized to be free of spectral interference. Fortunately, atomic spectral interferences are rarely encountered in the types of analyses done in most laboratories [35].

1.5.1.3.2. Physical interference

AS a result of variation in the physical characteristics, e.g., viscosity and surface tension, of sample and standard solution, interference in the condensed is experienced [38]. Different free-atom populations will result if sample and standard solutions vary greatly in bulk composition. Fortunately, viscosity and surface tension effects are negligible in the vast majority of analyses. Possible viscosity effects can usually be predicted by inspection of the solution, and are overcome by diluting the sample with water or a suitable organic solvent. If dilution is impracticable, perhaps because of the low concentration of the metal to be determined, the viscosity of sample and standard solutions should be matched by making up the standards to contain the same matrix as is present in the sample. It should be noted that excessive acid concentrations may increase sample viscosity sufficiently to cause interference. The use of organic solvents brings about an improvement in analytical sensitivity, probably because of the nebulizer of a greater proportion of small droplets in the sample spray than is obtained with aqueous solutions. Obviously, it is necessary to use the same solvent for both sample and standard solutions. Surface tension then effects may then ignore.

1.5.1.3.3. Ionization interference

Atoms of elements possessing very low ionization potentials become ionized at flame and furnace temperatures [39]. This interference is also known vapour phase interference; it reduces the free atoms population. Elements that commonly present a problem are Cs, Rb, K and Na. At nitrous oxide-acetylene flames temperatures Ca, rare earths, strontium and barium are also ionized to an appreciable extent. The magnitude of ionization in a flame depends on the concomitants present and their concentration.

1.5.1.3.4. Chemical interference

Chemical interference occurs when the analyte is contained in a chemical compound that is not broken down by the flame or furnace [39]. This results in the lower concentration of free analyte atoms than would occur in the absence of the interferent. Atomic absorption can only occur by free atoms. It is important to note that although higher temperature flames do successfully overcome some chemical interference; they do not alleviate all such problems. It is well known that common anionic species such as phosphate, sulphate and other oxygenated anions depress the absorption of the alkaline earth elements in the air-acetylene flame. The chemical compounds formed are relatively stable at the air-acetylene flame temperature and the formation of free atoms of the metals is partially obstructed. Various methods of counteracting this type of interference are available.

1.5.1.4. Methods of overcoming of chemical interference

A number of methods and procedures have been used to prevent or control chemical interference problem. They are described below.

1.5.1.4.1. Standard addition

Addition of an excess of the interfering anion to both sample and standard solutions overcomes chemical interference [39]. The method is simple but may reduce the analytical sensitivity of the element to be determined to below a useful limit. In this method a set of sample solutions are prepared to which are added available amount of the analyte element of interest. The idea is to maintain the matrix interference effect constant during the calibration procedure.

A difference in the slopes of the aqueous and standard addition graphs shows the prescence of interference which may be an enhancement or a depression of the analyte response. The disadvantage is that a set of solutions is needed for each sample (at least 4). The method will give accurate results provided no spectral interferences occur [39].

1.5.1.4.2. Matrix modification

The aim of this approach is to compensate for and eliminate the interference effect by chemical treatment [40]. A suitable chemical is added to the sample solution which either helps in removal of the interferant during ashing or masks the interference effect during the atomization stage. The most common type of matrix modifier is the use of a salt which prevents the loss of volatile analyte species during an ash stage and removes the interferent in electro thermal atomic absorption.

1.5.1.4.3. Matrix matching

In this case a set of standard solutions are prepared which accurately match the composition of the sample [40]. This procedure requires us to match the major components of the matrix. The level of interference, as in standard addition, is maintained constant in all standard and sample solutions.

1.5.1.4.4. Removal of the interfering ion

In this method the interfering ion can be removed by chemical or by ion exchange techniques [40].

1.5.1.4.5. Use a hotter flame

For alkaline earth elements this implies use of the nitrous oxide-acetylene flame [40]. Whilst the flame temperature is sufficiently high to completely overcome anionic interferences.

1.5.1.4.6. Addition of a releasing agent

The addition of releasing agent is probably the most commonly used technique [40]. A releasing agent is the salt of a metal which will form a stable compound with the interfering species. The releasing agent method is un successful only when the extent of the anionic interference is massive.

1.5.1.5. Atomic absorption literature survey

Ichinose et al., [41] described a method for determination of Cr (VI) as per chromic acid using solvent extraction technique then determined by atomic absorption spectroscopy. The atomic absorption determination of Cr (VI) can also be made more sensitive by extracting chromium as its pyrollidinedithio-carbamate [42, 43] or diethyldithiocarbamate [44] complex into methylisobutylketone (MIBK) and spraying the extract into the flame. However, Wang Hong et al., [45] decomposed the sample with mixed acid of phosphoric acid, sulphuric acid, nitric acid and perchloric acid, then the content of chromium was determined in nickel ore by flame atomic absorption spectroscopy.

Castillo et al., [46] mentioned a method for determination of chromium by flame atomic absorption using volatilization of metal trifluoroacetylacetonates. Solution of metal trifluoroacetylacetonate complexes of chromium are volatilized and then atomized in a heated silica tube connected directly to the generation apparatus. The complex vapor is carried to the atomizer by nitrogen. Also, Ottaway and Pradhan [47] discussed a method for determination of chromium in steel by atomic absorption spectroscopy with an air-acetylene flame and use 8-hydroxyquinoline as a releasing agent to suppress metallic interferences.

Zemberyova et al., [48] studied the interference effects and their elimination in the determination of chromium by flame atomic absorption spectroscopy in various types of soils and sludges from city water treatment using aqua regia and 2 mol dm^{-3} HNO_3. The authors also studied the interferences of Ca, Mg, Fe, Mn, Co and Ni in the chromium determination in these matrices and the effect of releasing agents NH_4Cl, Na_2SO_4, La $(NO_3)_3$, 8-hydroxyquinoline in the presence of the studied cations. On the other hand, Purushottam etal., [49] proposed ammonium bifluoride to suppress the interference arised during determination of chromium by atomic absorption spectroscopy. Ammonium bifluoride was found to be highly effective for suppressing the interference of diverse ions with Cr (VI) whereas a mixture of 1% ammonium bifluoride and 2% sodium sulphate was found suitable when both oxidations states are present. This method was applied on minerals, rocks, steels and alloys.

Stafllov and Zendelovska [50] developed a method for determination of chromium in iron minerals siderite ($FeCO_3$), hematite (Fe_2O_3), pyrite and marcasite (FeS_2) by flame and Zeeman electrothermal atomic absorption spectrometry. Iron in this method was extracted by using isoamyl acetate in hydrochloride acid solution and then chromium was determined in the inorganic phase.

Potgieter et al., [51] proposed a method for the selective extraction of Cr in cement matrix that based on treatment with 0.1M Na_2CO_3 solution and subsequent determination of chromium by electro thermal atomic absorption spectrometry.

The method has been applied to South African cement clinkers, natural gypsum, limestone and certified reference materials.

1.5.2. Spectrophotometric technique

Spectrophotometric method is a very important technique for determining metals in alloys, minerals and complexes, owing to its selectivity [52]. It offers the advantage of having calibration graphs that are linear over a wide range. Further improvement of the measurement came with the use of prism and grating monochromators for wavelength isolation. Photoelectric detectors were soon developed, but were quickly replaced with phototubes and photomultiplier tubes. The development of solid state microelectronics has now made available a wide range of detector type which are coupled with the computers, provide highly sophisticated readout electronic systems.

1.5.2.1. Basis of spectrophotometric technique

The basis of spectrophotometric methods is the simple relationship between the color of a substance and its electronic structure. A molecule or an ion exhibits absorption in the visible or ultra-violet region when the radiation causes an electronic transition in molecules containing one or more chromophoric groups. The color of a molecule may be intensified by substituents called auxochromic groups, which displace the absorption maxima towards longer wavelength. The color determining factors in many molecules is the introduction of conjugated double bonds by means of electron donor or electron acceptor groups [52]

The sequence of events in a spectrophotometer is as follow:

i) The light source shines through the sample.

ii) The sample absorbs light.

iii) The detector detects how much light the sample has absorbed.

iv) The detector then converts how much light the sample absorbed into a number.

v) The numbers are either plotted straight away or are transmitted to a computer to be further mainipulated (e.g. curve smoothing, base line correction).

1.5.2.2. Spectrophotometric chromium survey

A survey of literature revealed that a large number of reagents are suitable for the spectrophotometric determination of chromium. Kamburova [53] described a spectrophotometric method to determine of chromium ions. The method was based on the interaction of iodonitrotetrazolium chloride and tetrazolium violet with Cr (VI) and the formation of ion-associates with a 1:1 composition in hydrochoric acid medium.

Raj and Gowda [54] presented a method for the determination of chromium using thioridazine hydrochloride as a reagent. The reagent formed a blue coloured radical cation with Cr (VI) instantaneously at room temperature in (1-4M) orthophosphoric acid. The effects of acidity, time, temperature, order addition of the reagents and their concentrations were studied. The tolerance limit of the method towards various cations and anions associated with chromium were also reported. The blue species exhibited a coefficient absorption maximum at 640 nm with a molar absorption of 2.577×10^4 L. mol^{-1} cm^{-1}. As reported the method was successfully for the determination of chromium in steels.

Veena and Narayana [55] mentioned a procedure for the indirect determination of trace amounts of Cr (VI). The nitrite generated by the oxidation of hydroxylamine with Cr (VI) was used to form diazotize p-aminophenol. The diazonium salt formed was then coupled with isatin or isonipecotamide or frusemide in an alkaline medium to form azo dye.

Melwanki and Seetharamappa [56] developed propericiazine as a spectrophotometric reagent for the determination of chromium in environmental samples. Propericiazine formed a red colored radical cation, exhibited maximum absorption at 510 nm in H_3PO_4 medium. The method obeyed Beer’s law in the range of 0.15-2.25 mg/ml. The sandell’s sensitivity of the reaction was found 3.42 ng cm^{-2} .

Melwanki and Seetharamappa [57] used methdilazine hydrochloride reagent for the rapid spectrometric determination of Cr (VI) in environmental samples. The reagent gave a red colored complex in phosphoric acid medium. It exhibited maximum absorbance at 510 nm with sandell's sensitivity of 2.28 ng/cm^2 and Beer's law was valid over the concentration range of 0.1-1.9 mg/l.

Revanasiddappa and Kiran Kumar [58] reported citrazinic acid as new coupling agent for the indirect spectrophotometric determination of chromium by the oxidation of hydroxylamine in acetate buffer of pH (4.0-0.5) to nitrite. Molar absorptivity of the system was 2.12x10^4 L.mol^{-1}.cm^{-1} and sandell's sensitivity of the system was 0.00246 μg cm^{-2} at 470 nm. The color was stable for 6 hours and the system was obeyed Beer's law in the range 2.0-15 μg/mL of Cr (VI) in a final volume of 10 mL.

Revanasiddappa and Kiran Kumar [59] used trifluoperazine hydrochloride reagent for the rapid spectrophotometric determination of chromium. The method was based on the oxidation of trifluoperazine hydrochloride by Cr (VI) in the presence of H_3PO_4. The method was obeyed Beer's law in the range 1-18 μg/ml of Cr (VI) in a final volume of 10 mL. The method has been successfully applied to the analysis of the chromium in alloy steels and industrial effluents and pharmaceutical samples. The authors [60] used also Leuco xylene cyanol-FF as a sensitive reagent for the selective spectrophotometric determination of trace amounts of chromium in steels, industrial effluents and pharmaceutical samples. The method was based on the oxidation of leuco xylene cyanol-FF to its blue form of xylene cyanol-FF by Cr (VI) in H_2SO_4 medium pH (1.2 – 2.4). The absorbance of the formed dye was measured in acetate buffer medium pH (3.0 – 4.6).

Melwanki and Seetharamappa [61] used isothipendyl hydrochloride reagent for the simple spectrophotometric determination of Cr (VI). The reagent formed a red colored complex in H_3PO_4 medium. The red colored species exhibited an absorption maximum at 505 nm. The system was obeyed Beer's law at 2-18 μg/mL of Cr (VI) in a final volume of 10 mL. The molar absorptivity of the color system was 2.08x10 ^{4} L.mol^{-1} cm^{-1} and the developed color was stable for 2 hours.

Carvalho et al., [62] reported 4- (2- thiazolylazo) - resorcinol (TAR) reagent for the spectrophotometric analysis of chromium, whereas a red complex was formed at pH 5.7. The selectivity was improved by using EDTA and citrate as masking agents.

Rathore and Tarafder [63] explained a method to determine chromium in geological samples. This method based on the oxidation of hydroxylamine hydrochloride to nitrous acid by Cr (VI) in acetic acid medium. It followed by diazotization of the produced nitrite with *p*-aminophenylmercaptoacetic acid and subsequent coupling of the diazonium salt with *N*-(1-napththyl) ethylenediamine dihydrochloride in acidic medium to form a stable blueish azo dye. Beer’s law was obeyed in the range 0.02-10 μg /mL of Cr (VI).

Narayana and Cherian [64] studied a rapid procedure to determine trace amounts of chromium in alloy steels, industrial effluents, natural water samples and soil samples using variamine blue as a chromogenic reagent. The method based on the reaction of Cr (VI) with potassium iodide in acid medium to liberate iodine. The liberated iodine oxidized variamine blue to violet color. The developed complex had an absorption maximum at 556 nm. Beer's law was obeyed in the range 2-12 μg/mL of Cr (VI). The same authors used Azure B [65] as a chromogenic reagent for analysis of trace amounts of chromium. The method based on the oxidation of Azure B. Molar absorptivity and sandell's sensitivity of the system were found 3.77×10^{4} $L.mol^{-1}cm^{-1}$ and 2.76×10^{-2} μg cm^{-1} respectively.

Cherian and Narayana [66] reported saccharin as a new coupling agent for the spectrophotometric determination of Cr (VI). In this method Cr (VI) oxidized hydroxylamine in acetate buffer of pH 4.0 to nitrite, then diazotized p-nitroaniline or sulphanilamide to a diazonium salt. These diazonium salts were coupled with saccharin in an alkaline medium to form azo dyes. The method obeyed Beer's law in the concentration range of 1-16 μg/mL for chromium with *p*-nitroaniline-saccharin and 0.6-14 μg/mL of chromium with sulphanilamide-saccharin couples.

Fabiyi and Donnio [67] used variamine blue to determine of nano amount of chromium (VI). The method based on the reaction of Cr (VI) with potassium iodide in acidic medium to liberate iodine. The liberated iodine oxidized variamine blue to violet colored substances. It exhibited an absorption maximum at 615 nm. The method obeyed Beer's law in the concentration range of 0.05-0.45 μg mL^{-1}, molar absorptivity and sandell's sensitivity of $8.23x10^4$ L. mol^{-1} cm^{-1} and 0.0006 μg cm^{-2}, respectively.

SuvarDhan et al., [68] suggested a method based on the reaction 4-aminoantipyrine with 1-naphthol in the presence of oxidizing agent potassium dichromate in acidic medium to produce red colored product having an absorption maximum at

485 nm. The method was highly reproducible and has been applied to the analysis of chromium in synthetic, natural water samples and pharmaceutical preparations.

Chand Pasha [69] studied a rapid procedure to determine trace amounts of chromium in alloy steels, natural water samples, soil samples and pharmaceutical preparations using toluidine blue and safranine O as chromogenic reagents. The method based on the reaction of Cr (VI) with potassium iodide in acid medium to liberate iodine. The liberated iodine bleaches the blue color of the toluidine blue and the pinkish red color of the safranine O. The developed complexes had an absorption maximum at λ_{max} 628.5 nm and λ_{max} 532 nm. Beer's law was obeyed in the range 0.5 -12.4 and 0.4-13.4 μg/ml of Cr (VI), respectively.

Chapter (2) Experimental

2.1. Materials

All chemicals and reagents used were of the highest purity available. The following materials were used through the experiments carried out in the present work. Sodium hydroxide , sodium peroxide (Merck), Ammonium chloride, ammonium fluoride, sodium sulphate, sodium chloride (Adwic), calcium chloride, potassium chloride, magnesium sulphate, aluminum sulphate (Winlab), nickel sulphate (Chemie Loba), cobalt chloride, cupper chloride, zinc sulphate, ferric chloride (III), manganese sulphate and barium chloride (Merck).

2.2. Solutions

Different stock standard, reagents, buffer and sample solutions are prepared during the course of this investigation. Working concentrations from each element have been prepared in a fixed volume using double distilled water.

2.2.1. Reagents

2.2.1.1. Thymol blue (TB)

A stock solution of 1.0x 10^{-3}M thymol sulponphthalin was prepared by dissolving 0.0467 g of the reagent (E-Merck, Darmstadt, Germany) in 100 mL of ethanol (Adwic).

2.2.1.2. Dimethyl Yellow (DMY)

A stock solution of 1.0x 10^{-3}M dimethyl yellow was prepared by dissolving 0.0225 g of the reagent (Sigma-Aldrich Chemie GmbH) in 100 mL of ethanol (Adwic).

2.2.2. Potassium dichromate

A stock solution of 1.0x 10^{-4} M potassium dichromate was prepared by dissolving 0.00294 g of $K_2Cr_2O_7$ (Merck) in 100 mL of 0.01 M HCl.

2.2.3. Acids

Hydrochloric acid (Alderish), nitric acid (Poleskie), phosphoric acid and sulphuric acid (Adwic).

2.2.4. Acetate buffer

Acetate buffer solution was prepared [70] by add a volume of 0.1 M sodium acetate to a volume of 0.1 M acetic acid in 100 mL measuring flask to obtain pH range (2-6).

2.2.5. Standard stock solutions (1000 µg/mL)

All standard stock solutions were prepared by dissolving accurately weighted amounts of high purity reagents in suitable solvent [71, 72] and finally diluted to appropriate volumes and stored in clean bottles. Stock standard solutions of major and minor elements were prepared from the following salts, calcium chloride, magnesium sulphate, aluminum sulphate, nickel sulphate, manganese sulphate, barium chloride, cobalt chloride, cupper chloride, , ferric chloride and potassium dichromate. Standard solutions used for obtaining atomic absorption and spectrophotometric calibration curves were prepared by accurate dilution from stock solutions.

2.2.6. Sample dissolution procedure

0.1 g of powdered sample (-200 mesh) was digested with 1g of sodium hydroxide and 0.25 g of sodium peroxide in a nickel crucible. The mixture was mixed and fused for 30 min, then left to cool. The content was leached and dissolved with bidistilled water then filtrated and diluted to 50 mL standard flask.

2.3. Instrumentation

Thermo Electron Corporation S-series atomic absorption spectrometer model S-series in an air- acetylene flame was used during the course of this study. The atomic absorption spectrometer set up according to the manufacturer specification is outlined.

Adouble – beam Jasco (UV- Visible) spectrophotometer (Japan), model V-530 equipped with 10 mm matched quartz cells and connected to an IBM compatible computer.

2.4. Calibration and recommended procedure for atomic absorption technique

The flame parameters in atomic absorption technique were studied at different burner height (2.98, 3.78, 4.58, 5.38, 6.18, 7.78, 8.58, 9.38, 10.18, 10.98, 11.78, 12.58, 13.38, 14.18 mm) and different acetylene air flow (1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.8 L/min). All experiments were carried out at concentration level of 3 μg/ml of chromium. After adjustment of the parameters of atomic absorption spectroscopy to give maximum absorbance, standard absorbance values are measured for (0.25, 0.5, 1, 2, 3, 4, 5 and 6 μg/mL) Cr solutions and the calibration curve is constructed.

The effects of nitric, sulphuric, hydrochloric and phosphoric acids solutions on Cr signal were determined by atomic absorption method as follows: by preparing (0.25, 0.5, 1, 1.5, 2, 2.5 M) of different acid solutions under the study that contain 3 μg/mL of chromium. However, the effects of calcium, magnesium, iron and aluminum on chromium absorbance had determined by atomic absorption method as follows: by preparing 1000 μg/mL of each element under the study that contain 3 μg/mL of chromium.

2.5. Factors effecting on spectrophotometric studies

For spectrophotometric study, the effect of pH, volume of buffer, reagent concentration, sequence of addition, time and temperature parameters were examined.

2.5.1. Effect of pH

The effect of different pH values on the formation of complexes with metal ions under investigation was studied in suitable buffer media as optimum buffer for investigating the complexes. Aseries of solutions was prepared in which 1.0 mL of 1.0×10^{-4} M solution of metal ion, 2.0 mL of 1.0×10^{-3} M of reagent solution and 3.0 mL of buffer of different pH values in 10 mL measuring flask and completed with bidistilled water. The absorption spectra were recorded in the UV and visible regions against a blank of the pure buffer solution of the same pH value.

2.5.2. Effect of volume of buffer

To study the effect of volume of buffer on complex formation, the concentration of metal ion and reagent were kept constant at 1.0 mL of $1.0x10^{-4}$ M for metal ion and 2.0 mL of $1.0x10^{-3}$ M for reagent while the volume of buffer was regularly varied (1.0, 2.0, ……4.0 mL of the selected buffer solution) and the volume was completed with distilled water in case of TB reagent and with ethanol in case of DMY reagent to the mark of 10 mL measuring flask. The absorbance of each sample solution was measured at the recommended wavelength against reagent a blank.

2.5.3. Effect of reagent concentration

To study the effect of reagent concentration on the complex reaction, the concentration of metal ion was kept constant at $1.0x10^{-4}$ M while that of reagent was regularly varied (1.0, 1.5, ……3.5 mL of $1.0x10^{-3}$ M). 1.0 mL of the selected buffer was added and the volume was completed with bidistilled water in case of TB reagent and with ethanol in case of DMY reagent to the mark of 10 mL measuring flask. The absorbance of each sample solution was measured at the recommended wavelength against reagent a blank. The best reagent concentration can thus be determined.

2.5.4. Effect of sequence of addition

The effect of sequence of additions (metal, reagent, and buffer) on the complex formation was studied by measuring the absorbance of sample solution prepared using different sequences of additions against blank solution prepared by the same manner except of metal solution. The best sequence of addition was determined from the highest absorbance value.

2.5.5. Effects of time and temperature

The effects of time (5, 10, 15, 20, 25 and 30) and temperature (25, 30, 35, 40, 45, 50, 55 and 60) on the metal complexes of two reagents under consideration were studied by measuring the absorbance of the sample solution against reagent blank in the visible region at different time and temperature intervals. The highest absorbance values were obtained at the optimum time and temperature.

2.5.6. Effect of foreign ions

A systematic quantitative study of the influence of the foreign ions was investigated under the optimum experimental conditions except that the solution of foreign ions was added before the reagent solution and then the absorbance was measured.

2.5.7. Procedure for spectrophotometric determination of chromium (VI) using thymol blue reagent

3 mL of a stock solution 1.0x 10^{-3} M of thymol blue was added to 1mL of acetate buffer solution of pH 2.93 in 10 mL measuring flask containing 1 ml of 1.0x 10^{-4} M of Cr (VI) solution and completed with bidistilled water to the mark. The absorbance was measured at λ_{max} 548 nm against the corresponding blank similarly prepared without metal. A calibration graph for metal was constructed and the concentration

of unknown samples can be deduced by using such calibration graph.

2.5.8. Procedure for spectrophotometric determination of chromium (VI) by spectrophotometry using dimethyl yellow reagent

1 mL of acetate buffer solution of pH 3.76 was added to 3 mL of 1.0x10^{-3} M solution of dimethyl yellow in 10 ml measuring flask containing 1 mL of a stock solution 1.0x 10^{-4} M of Cr (VI) and completed with ethanol to the mark. The absorbance was measured at λ_{max} 484 nm against the corresponding blank similarly prepared without metal. A calibration graph for metal was constructed and the concentration of unknown samples can be deduced by using such calibration graph.

2.6. Molar ratio method

As the mole ratio method, described by Yoe and Jones [73] the concentration of metal ion was kept constant at 1.0 ml of 1.0x10^{-4} M while that of the reagent was regularly varied from

(0.2 - 3.0 ml) of 1.0 x 10^{-4} M. The absorbance of the solutions prepared was determined at optimum wavelength. The absorbance values were then plotted versus the mole ratio [Reagent] / [Metal]. The intersection of the two straight lines obtained showed the mole ratio of the most stable complex.

2.7. Continous variation method

In the present work, the modification of Jobs [74] continuous variation method performed by Vosbourgh et al [75] was utilized for investigating the reaction between metal ions and the reagent. A series of solutions was prepared by mixing equimolar solutions of the metal ion and the reagent in different proportions from (0.2 - 1.8 mL) of the same concentration while keeping the total molar concentration constant. A plot of the absorbance of the solutions measured at the recommended wavelength versus the mole fraction of the metal manifests a maximum at the expected mole ratio of the most stable complex. Shoulder probably indicates the formation of another type of complex.