ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY

Chromium Speciation Based on Selected Schiff's Bases: Attempted Investigations and Analytical Applications

Graduate Project (Chem. 774)

By
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Advisor
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July 2010

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ABSTRACT

Liquid-lquid extractive and UV-Vis spectrophotometric methods were attempted for speciation analysis of chromium. To do this, some laboratory-synthesized ligands (isatin-thiosemicarbazone, dihydrazine ninhydrin and ninhydrin semicarbazone) and a commercially available ligand, phenanthroline, ware used. There have been found that they are inappropriate for both analytical applications. However, the chemical properties may support some preconcentration or spectrophotometric applications. Suggestions for these possible applications are therefore forwarded for future studies.

Key words: liquid-liquid extraction, Spectrophotometry, chromium speciation, Schiff's bases

1. INTRODUCTION

Chromium was discovered in a mineral known as Siberian red lead. The mineral was first described in 1766 by German mineralogist Johann Gottlob Lehmann [1]. In 1797, Vauquelin studied the Siberian red lead, and he was able to isolate small metallic needles of chromium metal: The name of the element is derived from the Greek word "chroma", meaning color, because many of its compounds are intensely colored [1].

Elemental chromium has the symbol Cr and atomic number 24, first element in Group 6. It is a steely-gray, lustrous, hard metal, which takes a high polish and has a high melting point [2].

1.1 Chromium Occurrence

Chromium is the 21st most abundant element in Earth's crust. It exists primarily in the mineral chromite, which is present in soils, waters, rocks, and volcanic dust and gases [4]. After processing, chromium occurs in several chemical species [5]:

- *metallic chromium (chromium 0)*, which is mainly found in alloys, such as stainless steel, but also in chrome-plated objects.
- *trivalent chromium (chromium III)*, which exists in natural waters in hydrolyzed $Cr(H_2O)_4(OH)_2$ form and complexes, and even adsorbed on colloidal matter.
- *hexavalent chromium (chromium VI)*, which is found as CrO_4^{2-} , $HCrO_4^-$ or $Cr_2O_7^{2-}$, depending on the pH of the medium. It occurs in a range of compounds used in industrial processes.

The dominant Cr species generally depends on pH and Eh conditions (Fig. 1.1) [4]: in acidic soils ($pH<4$) it is $Cr(H_2O)_6^{3+}$, whereas at $pH<5.5$ it is its hydrolysis products, mainly $CrOH^{2+}$.aq; both these forms are easily adsorbed by macromolecular soil (eg. clay and humic acid) compounds [6].

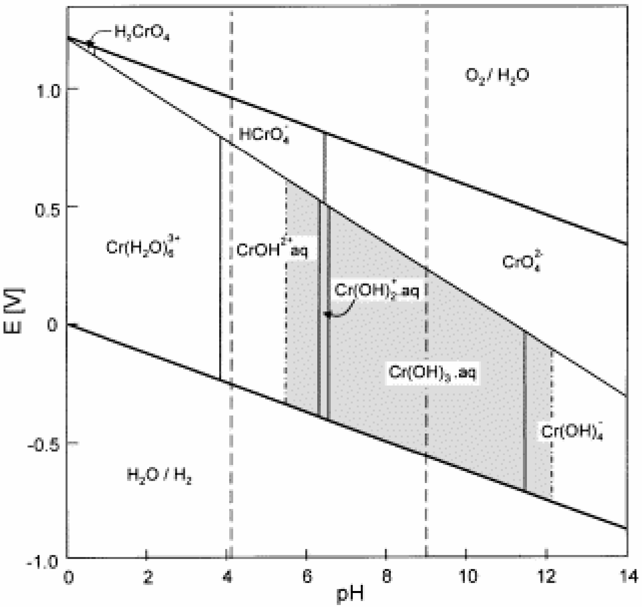

Fig. 1.1 Eh-pH diagram for chromium compounds in diluted aerated aqueous solutions in the absence of any reagents. Lined area shows the area of the occurrence of appropriate Eh and pH. The broken vertical lines represent the normal pH range in natural waters, whereas the grey region shows the domain of $Cr(OH)_3$ (s) stability for 10^{-6} M of total Cr.

1.2 Chromium Chemistry

Chromium chemical speciation (the kind of chemical species in which chromium appears) and the route of exposure are important factors in chromium toxicity [6].

1.2.1 Stable Oxidation States

Chromium exists in a number of oxidation states of variable stability. Chromium can exist in oxidation levels varying from -2 to +6, but in the environment chromium is mainly present with values: +3 and +6 [7]. The reduction potential diagram (Fig. 1.3) show that in solution, Cr(III) is the most thermodynamically stable species. CrO_4^{2-} is also relatively stable, but it has high positive reduction potential. Thus, it is a strong oxidizing agent and therefore unstable in acidic solutions in the presence reducing agents such as Fe^{2+} (from Fe(II)-containing minerals such as magnetite), H_2S, H_3AsO_3, and HSO_3^- or organic molecules with oxidizable groups (alkanes, alkenes, alcohols, aldehydes, ketones, carboxylic acids, mercaptans, and organic matters including microbial processes etc [1, 8].

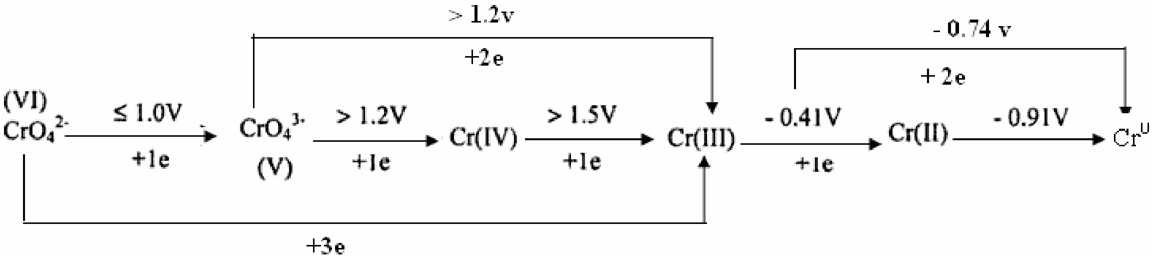

Figure 1.2 Reduction potential diagram for chromium. The standard electrode potential in volts (E^0) is indicated above the arrow linking two species and refers to rather concentrated solutions (standard state, of unit activity; ≥ 1 M); for more dilute solutions**,**

1.2.2 Oxidation-Reduction Chemistry

The redox transformation of Cr (III) to Cr (VI), and vise versa requires another couple (of oxidizing/ reducing agent) which accepts or gives the necessary electrons. In natural aquatic environments, the significant redox couples (reducing/oxidizing agents) are: H_2O/O_2, Fe (II)/Fe (III), Mn (II)/Mn (IV), S^{2-}/SO_4^{2-}, CH_4/CO_2 [1, 8]. Other factors also affect the oxidation-reduction characteristics of chromium, such as air temperature, humidity, and the contents of reducing agents [9]. Cr(VI) is stable in the more oxidizing environment with Eh higher than 0.6 V and pH above 6.0, whereas Cr(III) is thermodynamically stable at pH from 2 to 8 and Eh from +0.295 to - 1.0 V (Fig. 1). Eh-pH conditions directly affect the solubility of chromium compounds. Its solubility increases with acidity. In appropriate pH and Eh conditions and in the presence of reducing or oxidising agents, one form of chromium can transform into another [7].

Chromium(III) species are more prevalent under reducing conditions, and its release is increased only under strongly acidic conditions. At pH values greater than 9, the hydrolyzed Cr(III) species is negatively charged, demonstrating the amphoteric properties of Cr(III). Due to these, Cr(III) forms low-solubility compounds and adsorbs strongly on mineral surfaces and organic matter at pH > 4 [8].

Acid solutions of dichromate are strong oxidant [6]. During oxidation, Cr(VI) is reduced to Cr(III) through the formation of intermediates, Cr(V) and Cr(IV). The chromate ion in basic solution, however, is much less oxidizing as evident from E^0 values (Eq. 1.6–1.7) [10].

$$Cr_2O_7^{2-} + 14H^+ 6e \rightleftharpoons 2Cr^{3+} + 7H_2O, \quad E^0 = 1.33V \qquad (1.6)$$

$$CrO_4^{2-} + 4H_2O + 3e \rightleftharpoons Cr(OH)_3(s) + 5OH^-, E^0 = -0.13V \qquad (1.7)$$

Low concentrations of Cr(VI) are not stable in an acid solution, an observation which has implications with respect to the storage of Cr(VI) solutions in studies of environmental speciation. Thus, acidic Cr(VI) standard solutions and procedures for the determination of chromium in metal alloys and biological materials, which are based on acid dissolution of the sample in strong acid, followed by Cr(VI) determination using spectrophotometric or titrimetric procedures, must be used with caution due to this acid-induced reduction [11].

1.3 Chromium Toxicology

1.3.1 Chromium(III) as an Essential Nutrient

Trivalent chromium (Cr(III)) has been proposed to be an essential trace element animals, which may increase sensitivity to insulin and thus participate in normal glucose, cholesterol, fatty acid, carbohydrate and lipid metabolism [12, 13]. However, the evidence for Cr being essential is not definitive and sometimes controversial [14].

Its deficiency has been associated with impaired glucose tolerance, fasting hyperglycemia, glucosuria, elevated percent body fat, decreased lean body mass, maturity-onset diabetes, cardiovascular disease, decreased sperm count, and impaired fertility [15]. However, chromium is not an essential element for plants [13].

1.3.2 Chromium (VI) Toxicity

Certain forms of hexavalent chromium [Cr(VI)] are known respiratory carcinogens that cause of DNA damage through processes including, the intracellular metabolic reduction of Cr(VI) producing chromium species capable of interacting with DNA to yield genotoxic and mutagenic effects [16, 17]. It can also cause Cr(VI)-induced inflammatory/ immunological responses, cell transformation, respiratory cancers [18] and Cr(VI)-induced oxidative stress [19].

Consequently, US EPA has set the concentration of 0.1 mg L^{-1} of total chromium for drinking water as "maximum contaminant level goals", while US EPA, The European Economic Community and World Health Organization set drinking water guideline of values 0.05 mg/L for Cr(VI) [17].

1.4 Techniques for the Removal and Recovery of Chromium

Several techniques have been used for the environmental removal and recovery of chromium. These involve basic principle of chemical precipitation, electrochemical deposition adsorption, ion-exchange, complexation and magnetic separation [10, 20]. Some of which are well established methods that are discussed below.

1.4.1 Chemical Precipitation

The chemical precipitation method may involve a two step process. The first (for Cr(VI) step can be the reduction of Cr (VI) under acidic conditions (usually pH 2–3) to Cr(III), followed (the latter is less toxic, less soluble, and less mobile than Cr(VI) by the precipitation of Cr(III) hydroxide at a solution pH in the range 8–10 [8, 21]. Commonly used reducing agents are sulfur dioxide, sodium sulfite, sodium bi-sulfite and ferrous sulfate. In addition to iron (II) being a reductant, the iron (III) that is formed is an effective precipitant for Cr (III). This effect of iron(III) appears to be due to formation of insoluble ferric hydroxide which co-precipitates with Cr(III) hydroxide [10]. The process is shown in (Fig. 1.5).

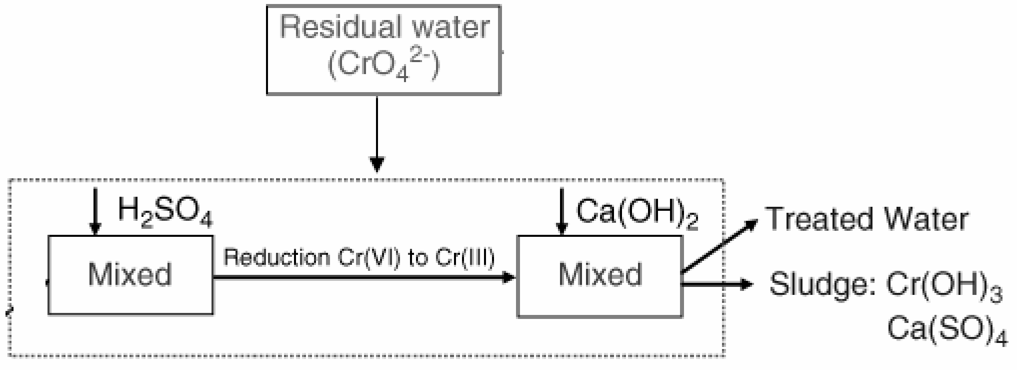

Fig. 1.3 Cr(VI) removal using chemical precipitation [22].

1.4.2 Electrochemical Precipitation

Electrochemical precipitation process (EPP) has been used to remove chromium from wastewater. The chromium removal efficiencies by such method are greater than 99% and the residual chromium concentration is less than 0.5 mgL^{-1}. Though chromium removal efficiency by such method is very high, it consumes huge amount of power. The effectiveness of this method for dilute solution is very low [8].

1.4.3 Adsorption

Surface adsorption is found to be important basis for the treatment of toxic element contaminated water. Numerous adsorbents such as activated carbon [23]**,** kaolinite, montmorillonite [24], zeolite [25, 26, 27] fly ash [28, 29], nanoporous materials Multi-walled carbon nanotubes [30], cement kiln dust [31] etc. are reported to be excellent adsorbent for chromium.

1.4.4 Bio-sorption and Bio-remediation

The process of heavy metal removal by biological material is known as bio-sorption. Many biomaterials such as seaweed, mold, micro-algae, fungi, bacteria, crab, shells, lignocelluloses solid waste, husk, sugarcane and various other agricultural products have been studied for their chromium binding abilities [32]. Various studies demonstrating bioremediation microbial reduction of chromate have been published recently [22, 33, 34, 35, 36].

The bioaccumulation process (biosorption) has advantages over conventional methods: first of all, it does not produce chemical sludges (i.e., is nonpolluting). Secondly, it could be highly selective. Moreover, it is more efficient and easy to operate. More over, it is cost effective for the treatment of large volumes of wastewaters containing low pollutant concentrations [22].

1.4.5 Liquid Membrane Technique

In recent decades, the use of liquid membranes for the separation and concentration of Cr(VI) has received considerable attention due to several advantageous characteristics such as commercial availability of selective extractants, high separation rates, possibility of metal recovery, etc. [21].

Several works have reported the use of amines and phosphorus compounds for the extraction of Cr(VI): phosphorus compounds such as tri-n-Octyl Phosphine Oxide (TOPO) [37], tri-n-butyl phosphate(TBP) [38], triphenylphosphine (TPP) [39]; tertiary amines such as tri-n-octylamine (TOA) [40] and Alamine 336 [41], and quaternary ammonium salts such as trioctylmethylammonium Chloride (Aliquat 336) [42].

In recent years, a remarkable progress concerning the industrial applications of liquid membrane systems is observed. The systems of membrane transport include [21, 43]: (1) bulk liquid membranes (BLMs), (2) emulsion liquid membranes (ELMs), and (3) supported liquid membranes (SLMs).

1.4.6 Electrokinetic

Electrokinetic extraction is a relatively new technique for the in situ remediation of polluted sites. The concept of the electrokinetic phenomena is based upon the relative motion of species under an electric field. The main mechanisms involved in the process are [43]:

- electromigration: the transport of ionic species in a wet porous medium towards the oppositely charged electrode

- Electro-osmosis: the motion of a fluid containing ions occurs in proportion to a stationary charged surface,
- Electrophoresis: there are charged particles moving relative to a stationary liquid.

1.5 Chromium Speciation Analysis

Speciation in chemistry is defined as distribution of an element amongst defined chemical species in a system**.** Speciation analysis is analytical activities of identifying and/or measuring the quantities of the different chemical forms of the elements present in a sample of one or more individual chemical species in a sample [45, 46].

Thus, speciation analysis of chromium usually consists of identification and quantification of various forms (species) of chromium in a given sample. It requires a multi-step approach, typically including sampling, sample storage, sample pre-treatment and instrumental analysis [47]**.** So, speciation analysis can be considered the last frontier in trace element analysis from the point of view of sensitivity and a challenge for method selectivity because it involves quantification of minor species and the discrimination of compounds of the same element [49, 49]**.**

The definition of species is based on several different levels of atomic and molecular structure where chemical forms of a same element are manifest. It can be considered differences at the levels of (1) isotopic composition, (2) electronic or oxidation state, (3) inorganic and organic compounds and their complexes, (4) organometallic species, and (5) macromolecular compounds and complexes [50].

1.5.1 Significance of Speciation Analysis of Chromium

In the past, the determination of total element concentrations was considered to be enough for clinical and environmental considerations. Although the total concentration of trace elements is still useful in many areas, the knowledge of speciation is of primary importance because the toxicity, mobility, bioavailability, and bioaccumulation depend on the nature of chemical species [49]. The toxicological potential of heavy metals and some metalloids is strongly determined by their oxidation state and /or the form of chemical binding. Moreover, bioavailability depends upon their special, species-dependent mechanisms of uptake or simply on solubility [51]. Thus, the importance of chromium speciation is governed by the fact that the toxicity and reactivity depend on the chemical form or oxidation state of chromium [52]. Speciation of chromium is most often reduced to the quantification of the two main oxidation states +3 and +6 [49].

Speciation Strategies

When strategies for the separation and individual determination of chromium species in solution are considered as a whole, it may be deduced that one or the other (or both) oxidation states of chromium may be selectively analyzed, while the other oxidation state requires an oxidation or reduction reaction prior to analysis [53].

Different strategies can be considered for non-chromatographic speciation and throughout this review we will discuss those based on: (i) different response yields obtained for different chemical forms of an element in the detector, (ii) selective extraction of species, (iii) derivatization procedures and (iv) selective volatilization among others [50].

1.5.2 Sample Pretreatment and Sample Preparation for Chromium Speciation

Direct determinations of chromium species in the real samples are difficult and limited with some instrumental techniques. For the determination of chromium species by many analytical techniques, sample pretreatment and separation–preconcentration procedures techniques have been used [54].

Filtration, acidification and extraction are common pre-treatment procedures. But for Cr, acidification affects not only particle size distribution but also induces severe matrix effects in speciation analysis, especially altering its oxidation state [47]. More over, experimental conditions may influence the reliability of the analytical results because of undesired Cr(III)-Cr(VI) interconversions [55]. Stabilization of the Cr(VI)/Cr(III) ratio can also be achieved at pH 9 whereas stable Cr(III)/Cr(VI) ratio could be ensured at nearly neutral pH, where oxidizing potential of Cr(VI) is too low to oxidize most reducers present in natural waters. [47].

To determine trace speciation of Cr in an environmental sample, a chemical separation and preconcentration step is often required prior to analysis. The separation methods mainly are coprecipitation separation [50], liquid-liquid extraction [46], solid-phase extraction [54], ion-exchange separation [53], ion chromatography [56], high-performance liquid chromatography [57], capillary electrophoresis [5], membrane extraction [58] and so on. However, chromium may be determined directly in real samples, without sample preparation, by applying electrochemical techniques [59], molecular absorption spectrophotometry [49].

Sample preparation for Cr speciation can be categorized in to two main categories: offline and online methods [4, 41].

In off-line methods, separation and pre-concentration of a particular Cr species are carried out before the sample insertion into the detection instrument. These methods have, however, a lot of disadvantages: they are complicated, time-consuming, resulting often in losses of the analyte and/or contamination appearance. Additionally, problems inherent to manual sample manipulation result in off-line methods not being really quantitative and their efficiencies are often low [41]

In on-line methods, the separation system is coupled with the detection system; as a consequence, separation, identification, and quantification of analytes are carried out in a one-step analytical process. These are preferable because they are relatively fast and require minimal sample pretreatment [56]**.**

1.5.3 Analytical Techniques for Chromium Speciation

The instrumental techniques for the trace determination of chromium may be divided into two main categories [59]:

(1) Speciation techniques, which enable the direct measurement of Cr(VI) in the presence of Cr(III), such as molecular absorption spectrophotometry or

voltammetry. These methods are based on a different ability of Cr(III) and Cr(VI) to form complexes.

(2) Non-speciation methods, based on the preliminary separation of both chromium forms, or on selective removal of a chromium species from the sample and subsequent, nonspecific measurement of the separated form by means of atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) or inductively coupled plasma atomic mass spectrometry (ICP-MS).

Cr speciation must rely on superior separation and detection capabilities, using methods of guaranteed accuracy and precision. Nowadays, there are varieties of techniques available for Cr analysis. Among them there are techniques which allow simultaneous determination of trivalent and hexavalent [60, 61] as well as methods involving separation and detection of one of these forms and the total Cr concentration [62, 63]. Usually, Cr speciation involves only differentiation between Cr(VI) and Cr(III) compounds; sometimes from the latter species those bound to organic ligands are separated, Another species is then determined using the same method after the residual chromium contained in the sample solutions has been reduced or oxidized [6, 7]**.**

The direct determination of chromium species in water samples can be done using valence-specific techniques, such as molecular absorption spectrophotometry [64, 65, 66] and voltammetry [67, 68]. On the other hand, valence-non-specific methods, such as flame atomic absorption spectrometry (FAAS) [69, 70, 71], graphite furnace atomic absorption spectrometry GFAAS) [72], and inductively coupled plasma atomic mass spectrometry (ICP-MS) [45], despite their feasibility to determine the trace or ultra-trace amounts, are not able to detect the chromium species. Thus, in encountering trace or smaller amounts of chromium, speciation studies require either a preliminary valence-specific separation/preconcentration process or selective removal of one of the species from the sample before the determinations [46]. Table 1.1 shows recent methods for speciation of chromium species.

Table 1.1 Recent Methods for Speciation of Chromium

Species & pH		Preconcentration reagent(s)	Sample	Analytical Technique	Ref.
Cr(III)	Cr (VI)				
	6	2-nitroso-1-naphthol impregnated alumina resin	Wastewater, tap water	FAAS	45
	7	Bis-[2-hydroxynaphthaldehyde] thiourea	Certified Reference material	CPE-FAAS	46
	1	1,4 Diaminoanthraquinone	Reference materials	UVVIS	64
	1M H_2SO_4	TBAI	Spiked water, electroplating wastewater	UV-VIS	65
	0.5M H_2SO_4	Diphenylcarbazide complex on an amberlite xad-4 resin column	Electroplating waste water	SPE-UV-VIS	66
6.1		Ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA)	Tap and river water	AdSV	67
	-	DTPA	Reference materials	CAdSV	68
	2	Cr(VI)- (APDC) chelate on MWNTs	Natural water	FAAS	69
7		8-hydroxyquinoline on surfactant-coated alumina on-line	Electroplating wastewater	FIA-FAAS	70
	7	Bis(2-hydroxy acetophenone)ethylendiimine loaded on activated carbon	River water milk	FIA-FAAS	71
7		2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol [5-Br-PADAP]	Vegetal samples	CPE-GFAAS	72
pH 3	pH 6	Immobilized nano-TiO_2	Drinking water	ETAAS	73
	Cr(VI)	Adsorption of Cr(III)–diphenylcarbazone complex on Amberlite XAD-1180 resin	Food, water, pharmaceutical samples	FAAS	74
Cr(III)		Bacillus thuringiensis var. Israelensis immobilized on Chromosorb 101	Red wine, rice, canned fish sea water, urine samples	FAAS	75
	-	Rhodamine B hydrazide	Drinking water, river water	Spectrofluorimetry	76

	pH 3	Aniline formaldehyde condensate (AFC) coated on silica gel	Wastewaters	FAAS	77
	1.2	Trioctylamine and Aliquat-336 by ELM	Pharmaceutical industry	UV-VIS	78
Cr (III)	Cr(VI)	HP Hypersil BDS C18	Certified reference material	Ion-pairing-HPLC-FAAS	79
	1–3	Tribenzylamine	Tannery effluents, electroplating waste water	UV-VIS	80
	0.5M HCl	ELM using TOPO	Tap water	FAAS	81
7		Alumina adsorbents-functionalized-purpurogallin	Underground water samples	FAAS	82
	Cr(VI)	DTPA and nitrate on the silver–amalgam electrode	Synthetic and river water	Hybrid algorithms voltammetric	83
2		Poly N-(4-bromophenyl)-2-methacrylamide-co-2-acrylamido-2-methyl-1-propanesulfonic acid-co-divinylbenzene	Tap water, lake water, spring water, wastewater samples	SPE-FAAS	84
7	1	Alumina-functionalized-isatin-thiosemicarbazone	Sea water, waste water	FAAS, UV–vis spectrophotometer,	85
	7	Silica gel chemically modified with niobium(V) oxide	River, tap and underground water	FIA-FAAS	86
	7	1-phenyl-3-methyl-4-benzoylpyrazol-5-one (PMBP)	Tap water, lake water	SPE-GFAAS	87
	5.5	Amberlite CG-50	Tap water well water s	FAAS	88
	4	ADPC	Soil sample	CPE-FAAS	89
7		2-nitroso-1-naphthol impregnated MCI GEL CHP20P resin	Sewage Sludge Bovine Liver certified reference	ICP-MS	90

Species		Preconcentration reagent(s)	Sample	Analytical Technique	Ref.
Cr(III)	Cr (VI)				
pH 3	pH 6	Immobilized nano-TiO_2	Drinking water	ETAAS	73

2 OBJECTIVES OF THE STUDY

2.1 General Objectives

The main objective of this study is:

- To investigate the analytical applications of selected Schiff's bases for chromium speciation using liquid-liquid and UV-Vis spectrophotometry.

2.2 Specific Objectives

The specific objectives of this study are:

- To determine properties of some Schiff's bases for liquid –liquid extraction,
- To study the spectrophotometric properties of the Schiff's bases after reaction with chromium species, and
- To spectrophotometrically determine appropriate Schiff's base for UV-Vis spectrophotometric speciation of chromium.

3 EXPERIMENTAL PART

3.1 Materials

SPECTRONIC Genesis 2pc UV-VIS Spectrophotometer (Germany), Stuart-SMP3 melting point apparatus, Oven, pH meter, Micropipette, magnetic stirrer with hot plate (Cole-Parmer Industrial Co.), Round-bottom-flask, Capillary tube, Solvent Jar, silica gel TLC plate, Volumetric flasks (25-ml, 50-ml, 100-ml, 200-ml)

3.2 Chemicals

Isatin and Thiosemicarbazide (Merck, Germany), Ninhydrin semicarbazone and Dihydrazine ninhydrin (laboratory-synthesized); Phenanthroline, K_2HPO_4, $K_2Cr_2O_7$ and $CrCl_3$ (BDH, England); Ethanol, Acetonitrile and methanol (Technopharm. Chem., India); KCl, NH_4OH and Acetic acid (Riedel-deHaen); Sodium acetate (Wagtech International Ltd.), KH_2PO_4 (Sigma –Aldrich, Switzerland), dichloromethane (Reagent Chem. Ltd.), Conc. HCl (Fizmark Chemicals, India); Deionized water (Milli-Q Millipore 18.2 $M\Omega cm^{-1}$ conductivity) was used for all dilutions. Laboratory glassware was kept overnight in a 10% (v/v) HNO_3 solution and then rinsed with deionized double-distilled water.

3.3 Synthesis of the Ligand, Isatin thiosemicarbazone (ITSC)

Synthesis of ITSC was done based on literature [85]. A mixture of isatin (2.0 g) and thiosemicarbazide (1.24 g) in water:ethanol (1:1, 140 ml) was heated under reflux for 1 hour. The colour of the solution gradually changed from orange to yellow. The mixture was cooled to room temperature overnight. The mixture was filtered slowly using suction filtration. Then, the yellow residue was washed repeatedly with deionized water. It was left to air dry for overnight. It was then put in to a desiccator.

To purify the synthesized compound, 2 g of isatin thiosemicarbazone was allowed to dissolve in heated 70 ml of ethanol, while 30 ml of ethanol alone was heated to boiling. The hot solution was filtered using suction filtration. The hot ethanol was for washing.

3.4 Determination of Melting Point of Isatin thiosemicarbazone (ITSC)

Small amount of ITSC was put into a capillary tube. Then, the tube was repeatedly dropped in long glass tube to load the ligand into the capillary tube. After adequate loading had been achieved, the melting point was determined using Stuart-SMP3 melting point apparatus. The melting point was found to be 246.7 ^{0}C, which is very close to literature value 243-245 ^{o}C [85] obtained after recrystallization in ethanol solvent.

3.5 Selection of Appropriate solvents: Extent of Solubility for the Ligands

Apppropriate solvent had to be selected as the compounds showed different solubility.

Table 3.1 Solubility tests for the ligands for selection of solvent.

Solvent	Solubility			
	ITSC	NSC	DHN	Phenanthroline
Acetonitrile	Very soluble	Very soluble	Very soluble	Very soluble
Chloroform	Slightly soluble	soluble	Slightly soluble	Soluble
Water	Slightly soluble	Slightly soluble	Slightly soluble	Slightly soluble
Ethanol	Soluble	Soluble	Soluble	Very soluble

Thus, acetonitrile was chosen as appropriate solvent for further applications for all ligands.

3.6 TLC Test for Purity

After series of experiments to identify appropriate solvent for TLC test, 10 % of NH_4OH in methanol solution was selected because of high polarity of the compound. A 10 % of this solution in dichloromethane was found suitable as a solvent (mobile phase).

A small solvent was put into solvent holder and waited for half hour for saturation of the solvent. Meanwhile, a small spot solution of ITSC and the reactants were applied on a TLC sheet. Then, it was carefully put into the holder. The plate was withdrawn when the solvent front reached the tip of the TLC plate. It was dried at room temperature. After this, the TLC was tested using UV radiation for spots. Only one spot was observed for the ITSC spot, which confirmed the purity of the compound. Thus, the synthesized compound was used without any extra treatment and test for further experiments.

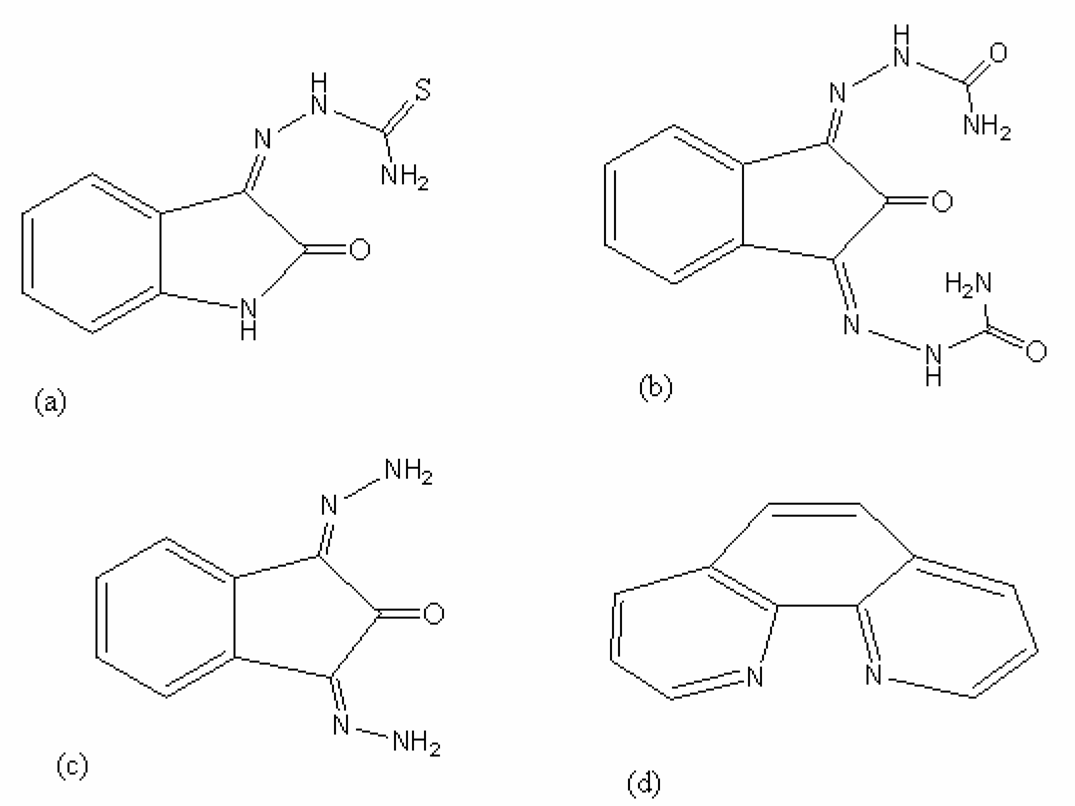

Fig. 3.1 Ligands studied for speciation of chromium: (a) Isatin Thiosemicarbazone, (b) Ninhydrin di-semicarbazone, (c) Ninhydrin-1,3-Hydrazone, (d) 1, 10-Phenanthroline

3.7 Preparation of Chromium Solutions

0.01 M stock solution of Cr(III) was prepared by dissolving of $CrCl_3 \cdot 6H_2O$, which was standardized by EDTA disodiumdihydrate. Stock solutions of 0.01 M of Cr(VI) were prepared by dissolving of $K_2Cr_2O_7$.

3.8 Preparation of Buffer Solutions

The complexation reactions were studied in buffer solutions, as complexation reactions of chromium species depends on pH of the solution. Table 3.2 shows

Table 3.2 pH buffer solutions and solutions used to prepare the solutions

pH	Solutions added in 200-ml
1	134 ml 0.2 M HCl and 50 ml 0.2 M KCl
2	13.0 ml 0.2 M HCl and 50 ml 0.2 M KCl
4	82 ml 0.1 acetic acid and 18 ml 0.1 ml sodium acetate
5	29.5 ml 0.1 acetic acid and 70.5 ml 0.1 ml sodium acetate
6	43.9 ml 0.1 M KH_2PO_4 and 6.1 ml K_2HPO_4
7	19.5 ml 0.1 M KH_2PO_4 and 30.5ml K_2HPO_4
8	50 ml 0.025 M and 20.5 ml 0.1 M HCl

3.9 Preparation of Solutions for UV-VIS Spectrophotometer and Reaction Test

For each experiment, 1 mM solutions were prepared by appropriate dilutions of the previously prepared 0.01 M solutions.

3.10 Method Design and Procedure

The following molecules, which proved to be complexing ligands including the well known phenanthroline were selected.

ITSC was reported to be chelating ligand on solid alumina for solid phase extraction [85], whereas DHN and NSC are synthesized and used extensively by inorganic chemistry students for their graduate projects. 1, 10-Phenanthroline is supplied commercially. In each case of the following, the pH of solution was adjusted by appropriate buffer solution. For Cr(VI), pH 1-4 and for Cr(III), pH 5-7 were found appropriate to study their properties (Table 1.1).

First, the reaction between a chromium species and a ligand were studied. If the reaction yields a hydrophobic complexation product, then the ligand could be useful for extraction, preconcentration and speciation studies. To do this, equal amounts of 0.01 M of chromium and ligand solutions were added in to a test tube. The mixture was left for some minutes. Then, any physical change (formation of a hydrophobic project in form of precipitation or suspension) was observed.

For UV-VIS spectrophotometric method, a mixture of equal amounts of 5 mM solutions of chromium and a ligand were added. The reaction mixture was left for a while. Then, it was scanned for UV-VIS spectrophotometry. The absorbance spectra of a chromium species, a ligand and their reaction mixture were studied. If distinct absorbance peaks (bands) be observed, one with least interferences from absorption bands of the metal, ligand and even the solvent would be carefully chosen.
The selectivity of the method over the other species would be carefully analyzed.

4 RESULTS AND DISCUSSIONS

Chromate (yellow color) has two ligand-metal charge transfer (LMCT) bands at wavelengths of 258 nm ($O^{2-}(\pi) \rightarrow Cr(3dt_{2g})$) and 349 nm ($O^{2-}(\pi) \rightarrow Cr(3de_g)$ [91]. Aqua complex of Cr(III) is a weakly-absorbing, too week to compare and interfere in the determination of distinct peak. Thus, its UV-Vis absorbance is not studied together with the ligand absorbances. More over, reaction mixtures of each chromium species with any ligand applied couldn't produce any hydrophobic species. Thus, their application for any known liquid-liquid extraction is concluded to be none.

4.1 Spectrophotometric characteristics of Chromium Species with ITSC

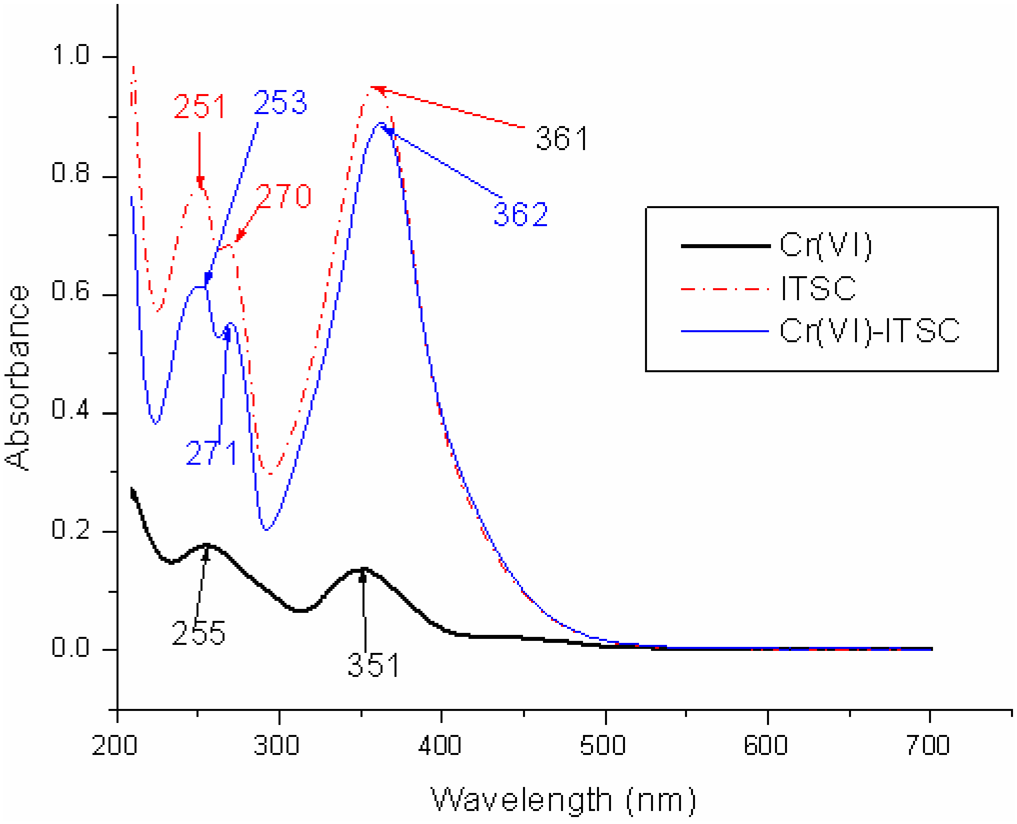

Fig. 4.1 UV-VIS Absorbances of Cr(VI), ITSC and Cr(VI)-ITSC

Some Schiff bases (eg. thiosemicarbazones) and others, having similar functionalities, have been reported to form chelates on solid sorbent materials. They were found to be effective for speciation analysis of chromium. For example, isatin thiosemicarbazone [85] and 2-pyridinecarboxaldehyde thiosemicarbazone [90] were used as chelating ligand for solid phase speciation extraction of chromium on alumina sorbent. Variation of pH was just sufficient to selectively adsorb either of the species.

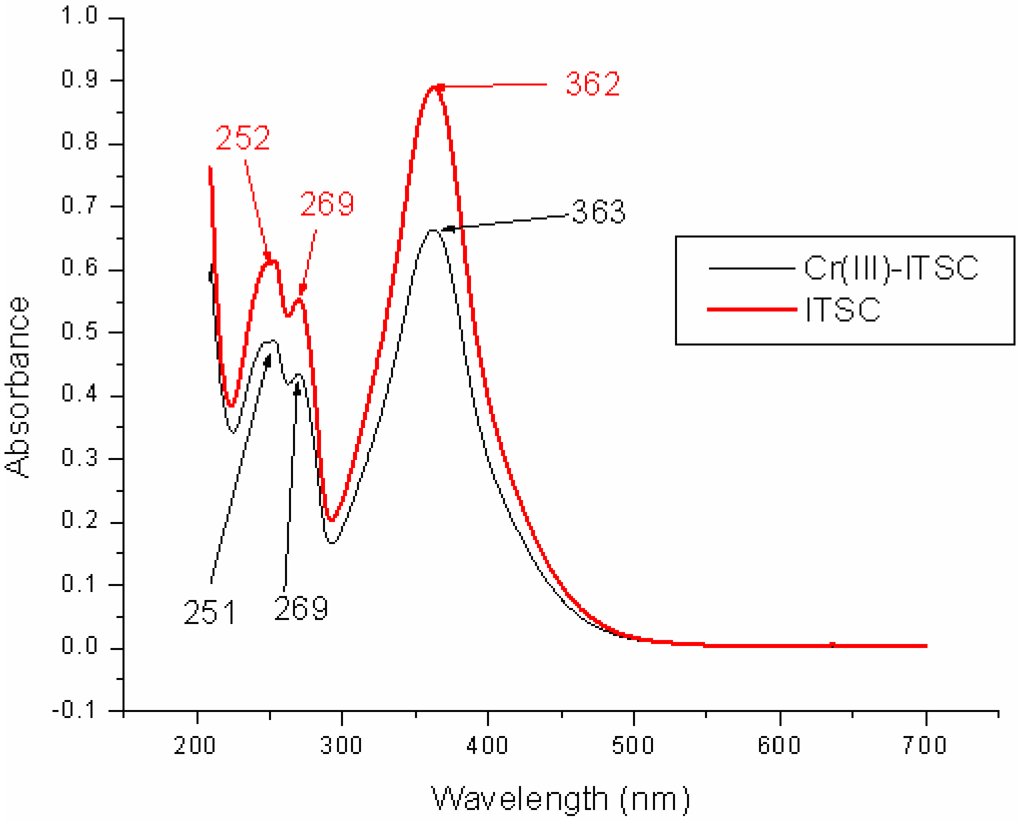

Fig. 4.2 UV-VIS Absorbances of Cr(III), ITSC and Cr(III)-ITSC

An attempt was made if isatin thiosemicarbazone could be applied liquid-liquid extractive separation and/ or spectrophotometric determination of either of chromium species. But, no formation of hydrophobic species was confirmed. UV data (Fig. 4.1 and Fig. 4.2) also provided inadequate information to confirm suitability of the molecule for spectrophotometric method. Moreover, the ligand is very strongly absorbing with a broadband in UV region so that distinct absorbances, if any, due to complex formation would be strongly interfered, which would make the method unreliable. Thus, ITSC could not be used as extraction and chromogenic reagent for both Cr(III) and Cr(VI) species.

Finally, the UV determination provided in based on ITSC on alumina sorbent in literature [102] must be the standard diphenylcarbazide method.

4.2 Spectrophotometric characteristics of Chromium Species with Ninhydrin di-semicarbazone

Ninhydrin di-semicarbazone (NSC), another Shiff base, was also tested. It has related functionality as ITSC. Moreover, it has two functionalities for chelating. It was reported that it can form complex with Ni [92].

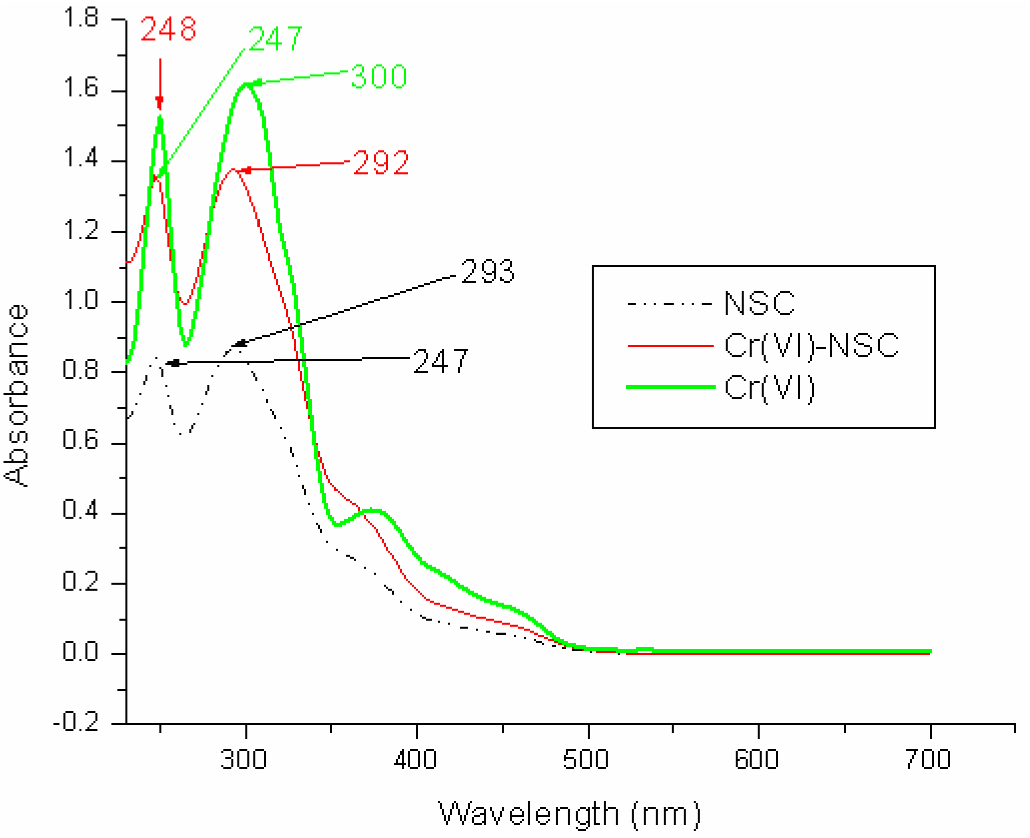

Fig. 4.3 UV-VIS Absorbances of Cr(VI), NSC and Cr(VI)-NSC

However, formation of hydrophobic complexes with both Cr(VI) and Cr(III) couldn’t be confirmed, and distinct absorbances couldn’t be achieved for both species. The absorbances for Cr(VI) case nearly coincide (Fig. 4.3). Avariation only at λ 370 nm was observed which suffered large interference from large absorption band of the ligand.

Normally, Cr(III)-aqua complex is kinetically very inert to react with some ('weak') ligands [31].

In fact, complex formation may require (be facilitated) by stirring and heating. Thus, no analytical application using liquid-liquid extraction and spectrophotometric method was found for NSC for speciation analysis of chromium. Despite these, due to its functional similarities as ITSC, it also may find extraction application for extraction based on its modification on sorbent materials. Thus, complexation can be archived only if strong ligands are used.

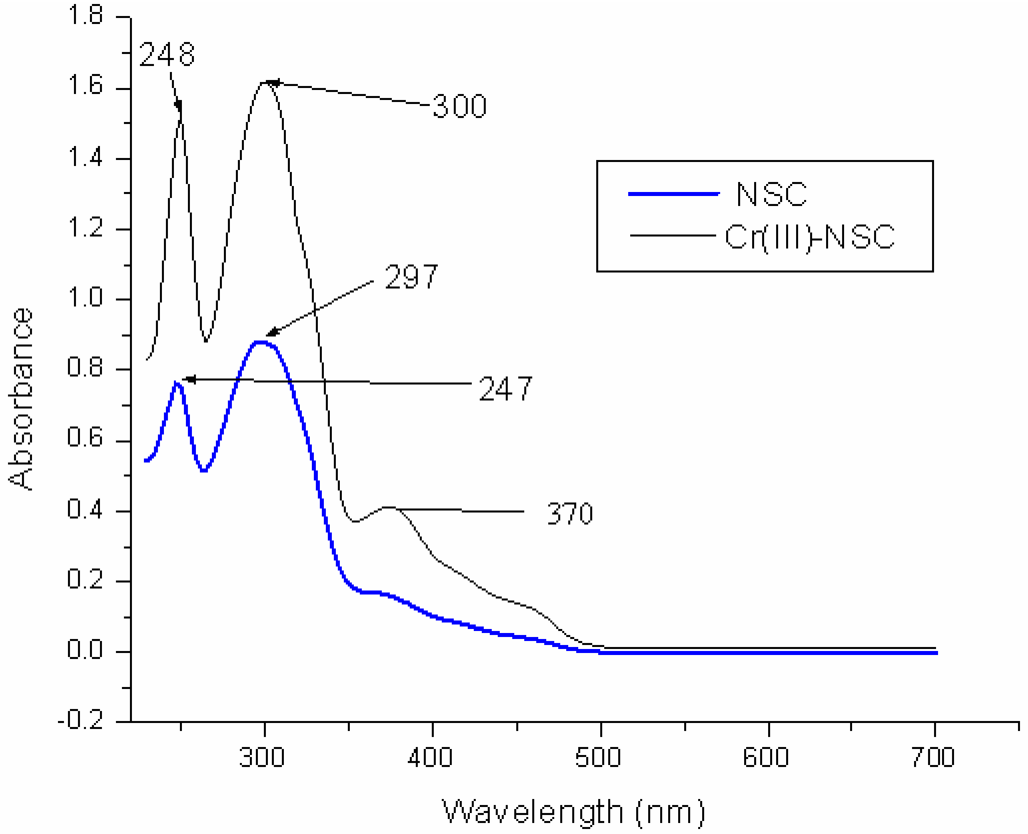

Fig. 4.4 UV-VIS Absorbances of Cr(III), NSC and Cr(III)-NSC

4.3 Spectrophotometric characteristics of Chromium Species with Ninhydrin 1,3- Dihydrazone (NDH)

The ligand has different functionality than the previous Shiff bases. For Cr(VI), the reaction resulted instant disappearance of colors of the mixture without formation of hydrophobic species. This could be rationalized as a reaction (most probably redox) between Cr(VI) and NDH occurred. This was confirmed in the UV spectra (Fig. 4.5) that some ligand peaks disappeared at 312 and 350 nm, and diminishing of Cr(VI) peaks at 258 and 350 nm. But, the disappearances were not helpful for spectrophotometry. Its application for liquid-liquid extractive chemistry is also none; however, similar mechanism was reported for spectroflourometry whereby Rhodamine-B Hydrazine (chromogenic compound) is synthesized from a nonchromogenic compound [93]. Rhodamine-B Hydrazine reacts with Cr(VI) to yield a product very selective and sensitive spectroflourometric method. Thus, the possibility of such mechanism for spectroflourometry must be studied.

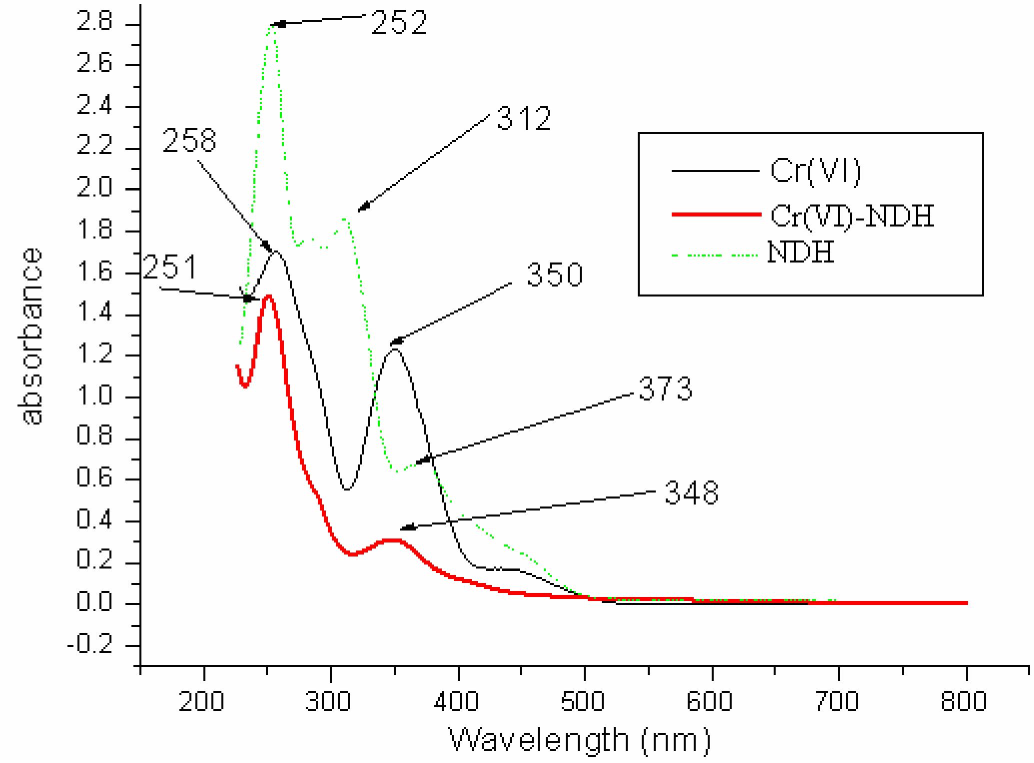

Fig. 4.5 UV-VIS Absorbances of Cr(VI), NDH and Cr(VI)-NDH

For Cr(III) reaction mixture, the absorption spectra for the ligand and Cr(III)-NDH mixture (Fig. 4.6) are almost overlapping; enhanced absorbance peak at about 274 nm is observed. But its peculiarity couldn't be reliable; therefore it can not have nay spectrophotometric application.

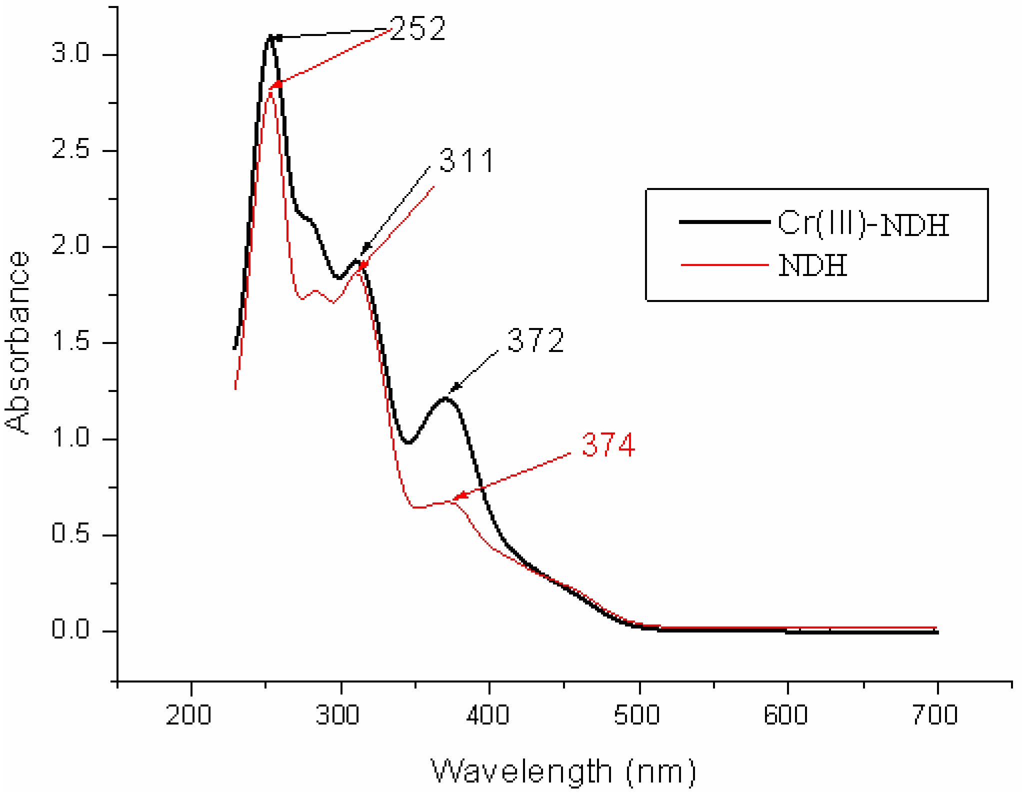

Fig. 4.6 UV-VIS Absorbances of Cr(III), DHN and Cr(III)-DHN

4.4 Spectrophotometric Characteristics of Chromium Species with 1, 10-Phenanthroline

1, 10-Phenanthroline couldn't form any hydrophobic species with any of chromium species. It is normally a complexing ligand for some cations; its application is practically (limited) to Cu, Ni and Fe species. The lone pair of theoretically a resonance stabilized. So, it is a weak electron donor, and it is unable to form complex with the relatively inert aqua complex of Cr(III) [18]. In addition to this, protonation of the ligand might be insufficient to form positively charged ligand, which subsequently would form a complex (ion-association) with anion of Cr(VI). Without any dilution factor, the two solutions of Cr(III) and Cr(III)-Phenanthroline absorbance bands just coincide (Fig. 4.7), which indicate the absorption peaks are merely of 1,10-Phenanthroline. But, in the case of Cr(VI) solution, there are changes in peak intensity and λ_{max} (Fig. 4.8). It is difficult to account the peak at λ_{max}= 270 nm for peak due to complex formation even if there is large shift in wavelength (about 9 nm)

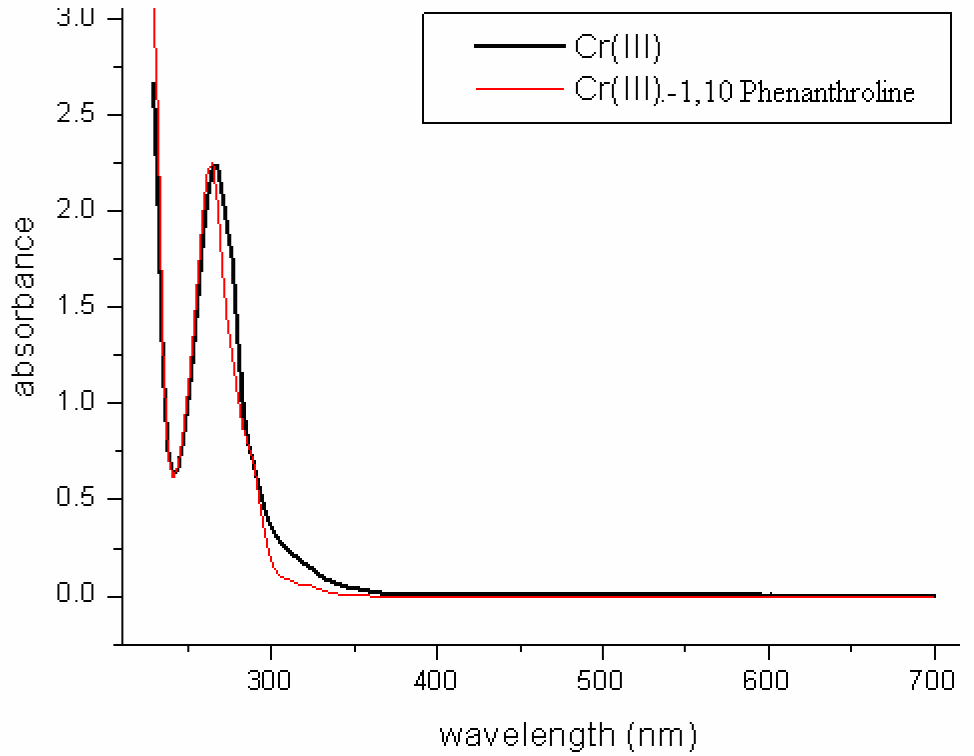

Fig. 4.7 UV-VIS Absorbances of Cr(III), 1,10-Phenanthroliine and Cr(III)-1,10-Phenanthroliine

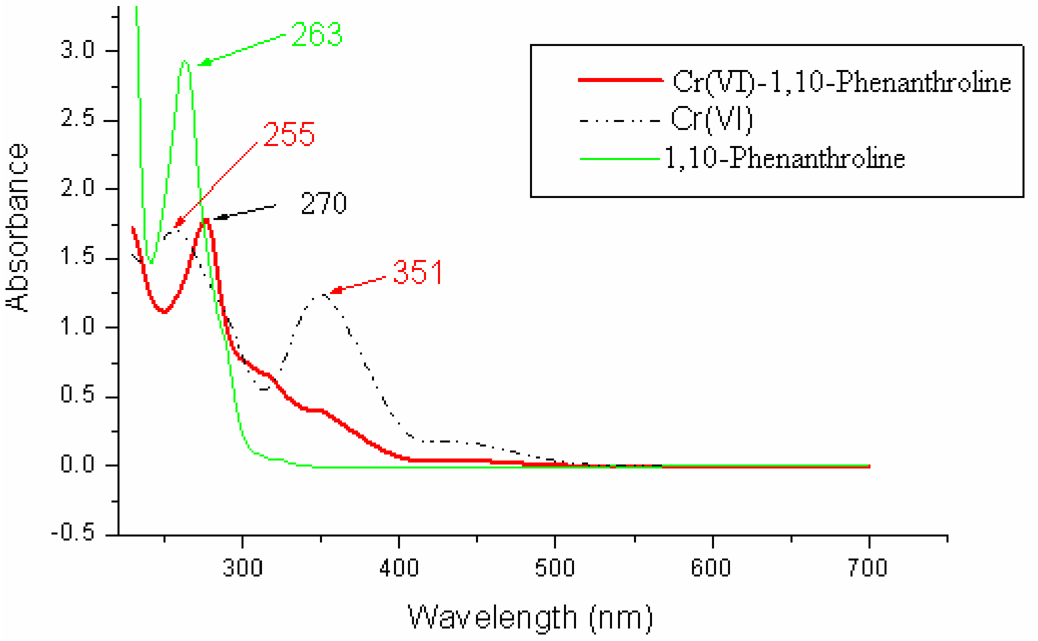

Fig. 4.8 UV-VIS Absorbances of Cr(VI), phenanthroliine and Cr(VI)-phenanthroline

5 CONCLUSIONS AND RECOMMENDATIONS

Despite a lot of reports are available for Schiff's base a excellent reagents for spectrophotometry and, preconcentration and speciation of chromium, these ones showed no characteristic property necessary for liquid-based preconcentration speciation and UV-VIS spectrophotometric determination under investigated conditions.

Isatin thiosemicarbazone was reported to be efficient for solid phase speciation and preconcentration of chromium species after it is physically adsorbed on alumina. But, its application for UV-VIS spectrophotometry, liquid-based preconcentration or speciation for chromium is not observed.

Ninhydrin di-semicarbazone, a semicarbazone with two chains of functionalities, was also confirmed that it did not fulfill similar requirements. But it may be useful for preconcentration and speciation studies based on modification of solid sorbents (such as alumina and silica gel). This possibility should be examined in the future.

Another unsuccessful result was obtained for 1,10-Phenanthroline.

Complexation reaction of ninhydrin-1, 3-dihydrazone with Cr(VI) also provided no positive result for UV-VIS spectrophotometer and liquid-based extraction; Only disappearances of color of solution and some absorption bands of the ligand were observed. However, it is understood that it may have a striking similarity of chromogenic property for spectroflourometry with Rhodamine-B hydrazine, because both have similar synthesis pathways and reactivies with Cr(VI). Thus, possible application in spectroflourometric method for the speciation studies must be checked.

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Declaration

This project is my original work. It is not presented for a degree in this and other universities. All resources and materials used for this project work are duly acknowledged.

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