ON-LINE PRECONCENTRATION, SPECIATION AND DETERMINATION OF CHROMIUM BY FLAME ATOMIC ABSORPTION SPECTROMETRY (FAAS) AND CHEMILUMINESCENCE (CL)

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Approval of the Graduate School of Natural and Applied Sciences.

_____
Prof. Dr. Canan ÖZGEN
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

_____
Prof. Dr. Hüseyin İŞCİ
Head of the Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

_____
Prof. Dr. Mürvet VOLKAN
Supervisor

Examining Committee Members

Prof. Dr. O. Yavuz ATAMAN (Chairman) _____

Prof. Dr. E. Hale GÖKTÜRK _____

Prof. Dr. İnci GÖKMEN _____

Prof. Dr. Sema BEKTAŞ _____

Prof. Dr. Mürvet VOLKAN _____

ABSTRACT

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Sürdem, Sedat

MS, Department of Chemistry

Supervisor: Prof. Dr. Mürvet Volkan

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Toxicological studies have shown that the degree of toxicity of some elements depends on the chemical form in which the element is present. Chromium (III) is considered as an essential micronutrient for human whereas chromium (VI) is a potentially carcinogenic agent. So the speciation of inorganic chromium in environmental samples is required for accurate assessment of pollution levels. The chromium

content in natural water is usually very low, and a preconcentration is often necessary prior to the determination.

A sensitive and selective preconcentration and speciation procedure is developed for the determination of trace and ultra trace amounts of chromium species by utilizing chemiluminescence (CL) and flame atomic absorption spectrometric (FAAS) techniques. The performances of amino silica-gel, amino sol-gel, mercapto silica-gel beads and metal oxides for solid phase extraction of chromium are examined either in column or batch type studies. Considering the advantage of concentrating Cr(III) and Cr(VI) ions separately simply by adjusting the pH of the medium, amino silica-gel resin is chosen in this study. The influences of different experimental parameters on the separation and preconcentration of chromium species such as pH, eluent concentration, flow rate, particle size of the resin are investigated.

Chemiluminescence detection studies are performed by using the catalytic effect of Cr (III) on the reaction between luminol and hydrogen-peroxide and Cr (VI) is detected after reduction to Cr (III). Luminol and H_2O_2 concentrations and the pH of the medium are optimized to increase the sensitivity of the system.

Chemiluminescence is inherently a very sensitive technique. When a preconcentration step is included in the CL mesurement of very low

concentrations of chromium is possible to determine. Indeed, a 25-fold enhancement in sensitivity of chromium ions is achieved after incorporating amino silane–gel colums in the system and 0.2 μg/L of chromium (corresponds to the concentration of chromium in natural waters) was measured.

A fully automated FI-CL system is designed that allows all necessary operations to be performed on-line. This system allows the pre-conditioning of micro-columns with different buffer solutions; adsorption of chromium species in micro-columns; washing these columns to remove interfering matrix components; elution of the species with minimum volume; transporting the species and chemiluminescence reagents to the cell; and, finally, cleaning of all pertinent conduits in the FIA-system in order to prevent carry-over between individual samples.

Keywords: Atomic Absorption Spectrometry, Chemiluminescence, Flow Injection, Chromium, Preconcentration, Speciation.

ÖZ

ALEVLİ ATOMİK ABSORPSİYON SPEKTROMETRİ VE KEMİLUMİNESANS İLE KROMUN HAT ÜSTÜ ÖNZENGİNLEŞTİRME, TÜRLENDİRME VE TAYİNİ

Sürdem, Sedat

Yüksek Lisans, Kimya Bölümü

Tez Yöneticisi: Prof. Dr. Mürvet Volkan

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Toksikoloji çalışmaları bazı elementlerin toksik derecelerinin o elementin bulunduğu kimyasal formuna bağlı olduğunu göstermektedir. Cr (III) insanlar için zorunlu mikro-besin olarak kabul edilirken, Cr (VI)'nın potansiyel kanserojen olduğu bilinmektedir. Bu yüzden, kirlilik derecesinin doğru değerlendirilebilmesi için çevresel örneklerdeki inorganik kromun türlendirilmesi gereklidir. Doğal sulardaki krom içeriği genellikle çok düşük olduğu için çoğu zaman tayinden önce bir önzenginleştirme zorunludur.

Eser miktarlardaki krom türleri tayini için kemiluminesans ve alevli atomik absorpsiyon spektrofotometrik teknikleri kullanılarak hassas ve seçici bir önzenginleştirme ve türlendirme yöntemi geliştirilmiştir. Amino silika- jel, amino sol-jel, merkapto silika-jel parçacıkları ve metal oksitlerin katı faz çıkarma performansları kolon ya da kesikli süreç tipi çalışmalarla incelenmiştir. Cr (III) ve Cr (VI) iyonlarının ayrı ayrı, ortamın asitliğini basitçe ayarlamak suretiyle derişitilmesi avantajı göz önünde bulundurularak amino silika-jel reçinenin bu çalışmada kullanılmasına karar verilmiştir. pH, eluent derişimi, akış hızı, reçinenin parçacık büyüklüğü gibi çeşitli deneysel parametrelerin kromun ayırılma ve önzenginleştirilmesinin üzerindeki etkileri incelenmiştir.

Kemiluminesans ölçüm çalışmaları Cr (III)' ün luminol ve hidrojen peroksit ile reaksiyonu üzerindeki katalitik etkisinden yararlanılarak yapılmıştır. Sistemin duyarlılığını arttırmak için luminol, hidrojen peroksit derişimlerive ortamın asitliği en uygun duruma getirilmiştir.

Kemiluminesans temelden çok duyarlı bir tekniktir. Kromun kemiluminesans ölçümünde bir önzenginleştirme aşaması eklendiğinde çok düşük derişimlerin tayini mümkün olmaktadır. Sisteme amino silika-jel kolonları eklendikten sonra sistemin duyarlılığında 25 kat artış elde edilmekte ve 0.2 µg/ L krom derişimi (doğal sulardaki krom derişimine karşılık gelmektedir) ölçülebilmektedir.

Bütün gerekli işlemleri doğrudan yapabilmeyi sağlayan, tamamen otomatik bir akışa enjeksiyon-kemiluminesans sistemi tasarlanmıştır. Bu sistem, mikrokolonların değişik tampon çözeltileriyle önkoşullandırılması; krom türlerinin mikrokolonlara tutunması; kolonların yıkanarak girişim yapan matriks bileşenlerinin uzaklaştırılması; türlerin minimum hacimle toplanması; türlerin ve kemiluminesans reaktiflerinin hücreye taşınması ve sonuç olarak, akışa enjeksiyon sistemindeki tüm ilgili kanalların birbirinden ayrı örneklerin aralarında karışmasını engelleyecek şekilde temizlenmesine imkan vermektedir.

Anahtar Sözcükler: Atomik Absorpsiyon Spektrometre, Kemiluminesans, Akışa Enjeksiyon, Krom, Önzenginleştirme, Türlendirme.

To My Son

Coming Soon...

Erdem Erbil SÜRDEM

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TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

CHAPTER 1

INTRODUCTION

1.1 Occurrence and Recovery of Chromium

The two primary oxidation states of chromium in natural waters, the trivalent state Cr^{+3} (Cr (III)) and hexavalent state Cr^{+6} (Cr (VI)), differ significantly in biological, geochemical and toxicological properties. Thermodynamic calculations indicate that almost all of the chromium exists as Cr (VI) in seawater. Cr (III) compounds are sparingly soluble in water, while Cr (VI) compounds are readily soluble which may result in enhanced levels of Cr (VI) in water sources (1).

Chromium is a naturally occurring element found in rocks, animals, plants, soil, volcanic dust and gases. Humans are exposed to chromium when eating food, drinking water and inhaling air. The average daily intake from air, water, and food is estimated to be 0.001-0.003 µg, 2 µg and 60 µg respectively. The reference dose (RfD) for Cr (III) is 1 mg/kg per day and the RfD for Cr (VI) is 0.005 mg/kg per day (1).

Dermal exposure to chromium may occur during the use of products that contain chromium such as wood treated with copper dichromate or leather tanned with chromic sulfate. Occupational exposure to

chromium occurs from chromate production, stainless-steel production, chromate plating, and the tanning industries (1).

Occupational exposure can be two orders of magnitude higher than exposure in the general population. People who live in the vicinity of chromium waste disposal sites or chromium manufacturing and processing plants have a greater probability of elevated chromium exposure than that of the general population (1).

1.2 Toxicology and Property of Chromium Species

Industrial development has brought many problems of environmental pollution in its wake. Therefore, water-quality monitoring in process effluents has become extremely important. Toxicological studies have shown that some essential and non-essential elements become toxic at certain level of concentration; the degree of toxicity of some elements depends on the chemical form in which the element is present. Consequently, chemical speciation has attained an increased significance and, in this context, it is particularly of interest to develop automated procedures for determining chromium (2).

In aqueous solution, chromium exists in the Cr (III) and Cr (VI) oxidation states. The properties of these species are widely different. According to Mennen (3), insulin hormone could not work without the presence of Cr (III). Insulin is the master hormone of the metabolism that controls not only the blood-sugar levels and many other aspects of carbohydrate breakdown and storage, but also directs many of the

metabolism involving fat, protein and energy. Chromium is essential as a trace element for the ordinary life of living organisms (50-200 µg per day is necessary). Hexavalent chromium is known to have toxic effects on biological systems (4); it can in particular be a cause of DNA damage, cancer, or allergic reactions (5). As a matter of fact, Cr (VI) is not genotoxic in itself, but its redox behavior produces species that are potentially toxic. The general mechanism of Cr (VI) activity in the cellular and subcellular systems postulates that Cr (VI) under physiological conditions (pH=7.4) penetrates easily into cells, being transported by sulfates or phosphates, considering that the tetrahedral CrO_4^{2-} and pseudo tetrahedral $HCrO_4^-$ ions have structural similarity with the biologically important inorganic anions SO_4^{2-} and PO_4^{3-} (6).

In cellular media, chromium compounds can produce cellular effects along two distinct pathways that are related though independent of each other (6). The first one is an indirect pathway where Cr (VI) undergoes intracellular reduction towards more stable trivalent species, producing reactive Cr (V) and Cr (IV) intermediates, simultaneously with the generation of reactive oxygen species and other free radical species. Cr (VI) reduction by glutathione generated both reactive Cr (V) and a glutathionethyl radical and caused direct damage in the form of glutathione-Cr-DNA complexes. In contrast to this glutathione reduction process, the reaction of Cr (VI) with H_2O_2 produced superoxide anion and/or hydroxyl radical, and led solely to breaks in the ROS-mediated DNA strand and 8-oxo-2' deoxyguanosine (4-5).

The second pathway, by direct Cr (III) binding to DNA and/or nuclear proteins, consists mainly in DNA-DNA and DNA-protein cross-links and, to a lesser extent, in breaks in the single DNA strands (4-5).

1.3 Speciation

Because of the different toxicities and bioavailability of Cr (III) and Cr (VI), determination of total chromium content does not give full information about possible health hazards. Hence monitoring of the concentration of the separate chromium species is of great importance. The specification of inorganic chromium in environmental samples is required for accurate assessment of pollution levels (7).

Speciation techniques based on the separation of different chromium species before detection possess the advantage of making possible combination of selective detection techniques with highly sensitive ones. Although the detection limit of the combined technique can be improved by a preconcentration step, a serious drawback is the complex and time-consuming sample pre-treatment, which may include solvent extraction (8,9), coprecipitation (10,11), electrochemical separation (12), ion exchange (13,14) and solid-phase extraction (15).

Among the numerous methods developed for chromium speciation, those which separate the individual species physically followed by a direct quantitation are preferred because they are relatively fast and require minimal sample pretreatment. The latter factor is particularly

important because prolonged sample manipulation may affect the distribution of the chromium species significantly (16, 17).

The predominant trend in recently proposed methods for speciation of chromium is the use of liquid chromatography and flow methods of analysis. Coupled methods combining liquid chromatography with AAS detection, inductively coupled plasma, mass spectrometry and visible spectrophotometer have been developed. Ion chromatography (18), reversed-phase liquid chromatography (19) and ion-pairing chromatography (20) are usually applied as the chromatographic methods. Reversed-phase C_{18} silica gel has been used for the chromatographic separation of chromium species. Inconveniences of the chromatographic methods are the complicated procedures and long analysis times. In some cases the resolution of the two oxidation forms is poor.

1.4 Pretreatment Techniques for Chromium

The most frequent pretreatments used are preconcentration by column/ion-exchange and complex formation. Other treatments such as precipitation, filtration, centrifugation and microwave digestion are the techniques used before detection and these treatments are commonly used with preconcentration on column or complex formation (21).

1.4.1 Preconcentration on Column

Extending an analytical system for the determination of chromium samples of low contents, such as those found in drinking water and sea water, generally requires a preconcentration step (21).

The most frequently used pretreatment in literature on atomic spectrometric techniques is preconcentration on columns, which permits the retention and subsequent elution of one or two species. In some cases, one of the two species is precomplexed before separation in a column. The pretreatments belonging to preconcentration on column are the most common in all the techniques, except in graphite furnace AAS, where complex formation is mainly used (21).

1.4.1.1 Preconcentration of Chromium (VI)

Most methods of selective extraction are based on the extraction of Cr (VI) with chelating agents such as dithiocarbamates (22) or liquid ion exchangers, such as amberlite IRA-400 (23). The main difficulty with this type of separation concerns the use of acidic media for the quantitative extraction of Cr (VI) which favors its reduction, especially in the presence of organic materials. Preconcentration of chromium (VI) has been carried out using different types of columns which are listed in Table 1.1 and in Table 1.2.

1.4.1.2 Preconcentration of Chromium (III)

Preconcentration methods based on the formation of Cr (III) complex, have not been applied much because of the inert nature of hydrated Cr (III) species. Cr (III) concentration is usually calculated by difference after the determination of the total chromium and Cr (VI) (24). However, in some cases of analysis of natural waters this determination may not be adequate, owing to the large uncertainties introduced by such a calculation (25).

Much work has been investigated in developing on-line preconcentration procedures for determining Cr (VI) than Cr (III) due to the inertness of Cr (III) which hinders efficient complexation. However, on-line preconcentration of Cr (III) was recently achieved by means of solid sorbent extraction using resins with different immobilized functional groups, or by employing chelating ion-exchange columns (7). Preconcentration of chromium (III) has been carried out using different types of columns shown in Table 1.1 and in Table 1.2.

Table 1.1 Column packing materials used for Cr (III) and Cr (VI)

Cr (III)	**Cr (VI)**
Chelating ion-exchange resin with salicylic acid functional groups (**26**)	Melamine formaldehyde resin (**50**)
Chelating resin PAPHA (**27**)	C18 bonded silica reversed phase sorbent with DDTC as the complexing agent (**51**)
Quinoline-8-ol or Muromac A-1 resin (**28, 29**)	Chromabond NH_2 (**52**)
Polystyrene-divinylbenzene resin (**30**)	Phosphate treated sawdust as adsorbent (**53**)
Sephadex column (**31**)	ZnO (**54**)
Reversed phase C18 column (**32**)	Alumina micro-column (**55 ,56**)
Anion exchange columns of MonoQHR 5/5 (**33**)	Anion-exchange resins (**57**)
Ion-exchange column of Muromac A-1 (**29**)	Amberlite diluted in MIBK (**58**)
Ion-exchange Bio-Rad AGMP-1 after complexation with sulfonated azo-dyes (**34**)	Liquid anion exchangers such as Amberlite LA-1 or LA-2 (5**9**)
Chelex-100 or Lewatit TP 207 (**35**)	Dowex 1-X8 anion exchange resin (**60**)
Polyacrlamide (3**6**)	Polyimine Detata sorbent (**61**)
Polyacrylamidazone-hydrazide lacmoid chelating fiber (**37**)	Polystyrene divinyl benzene resin functionalized with 2-naphthol-3,6-disulfonic acid (**62**)
Synthetic ettringite (**38**)	Polyether ether ketone (PEEK) knotted reactor (**63**)
Amberlite IR-120 cation-exchange resin (**39**)	Amberlite IRA-400 cation-exchange resin (**39**)
C18 with PHP (**40**)	Eurospher 100-C18 (**40**)
Chelex 100 anion-exchange resin (**41**)	AG-MP-1 anion resin (**41**)
Cellulose with Cellex P (**42**)	Cellex T (**42**)
Anionite AV-17-8 (**43**)	Cationite KB-4 (**43**)
IDAEC chelating cellulose microcolumn (**44**)	DEAE chelating cellulose micro-column (**44**)
Aromatic sulfonic acid silane column (**45**)	Bio-Rad 1-X4 column (**45**)
PAAO resin (**46**)	PDTC resin (**46**)
IC-PAKA anion exchanger column (**47**)	IC-PAKA anion exchanger column (**47**)
Dealginated seaweed cation exchanger (**48**)	Dionex CS5 anion exchanger column (**64**)
Prosep-chelating I resin (**49**)	PTFE (**65**)

Table 1.2 Column packing materials used for both Cr (III) and Cr (VI)

Cr (III) & Cr (VI)
Strongly basic anion exchanger with H_2SO_4 (**66**)
Dionex AG4A (**67**)
DEAE-Sephadex A-25 (**68**)
Silica gel loaded with anion exchanger Adogen 464 (**69**)
Polyacrylonitrile sorbent modified with polyethylene polyamine (**70**)
C18 (**71**)
Eurosphere 100-C18 (**72**)
Eurosphere PRC18 (**73**)
Dionex CS5 or Dionex ASH (**74**)
Anion-exchanger Omni Pac PA100 (**75**)
Dionex Ion Pak A57 (**76**)
Anion exchanger Ion Pac A65 (**77**)
Excelpak ICS-A23 (**78**)
Polyspher IC AN anion exchanger (**79**)
Activated alumina micro-columns (**80**)
Methyltrioctylammonium chloride-loaded silica gel (**81**)
Polymeric Detata sorbent with amino carboxylic groups (**82**)

1.5 Analytical Techniques for Determination of Chromium

The most common techniques used are UV-VIS spectroscopy (83), atomic absorption spectroscopy (84), and electrochemical techniques (85). The latter ones, on the basis of an original setup introduced by Turyan (86), make the detection of Cr (VI) possible at a level as low as one part per trillion. Gas chromatography is also a technique used in the detection of Cr (VI) since it forms a volatile chelate (87). These techniques are well adapted to the analysis of liquid samples. When solubilization step is required these techniques are not convenient as they do not always provide the accurate ratio and concentration of Cr (III) and Cr (VI) in the sample.

To overcome the problem of chromium valence ratio alteration by pretreatment operations, *in situ* techniques such as Raman (88) or X-ray absorption spectroscopy (89) have been developed. These techniques require solid samples without pretreatment and therefore provide results that are more representative of chromium valence in the original sample. However, these techniques require traditional synchrotron installations and cannot be used for routine work. Furthermore, the cost of experimentation is very high.

More recent developments allow both detection and quantification of the two chromium species without separation step. In particular, capillary electrophoretic techniques (90), ion chromatography analyses (91), HPLC, combined with direct injection nebulization and ICP-MS (92), and reverse phase-HPLC coupled to various spectrometric detection methods (GFAAS, ICP-MS) (93) make the simultaneous differentiation of chromium species possible.

Inductively coupled plasma atomic emission spectrometry (ICP-AES) and ICP mass spectrometry (ICP-MS) are the favored techniques of detection used in speciation studies because of their generally higher sensitivity over atomic absorption spectrometry (AAS) (94). However, the sample introduction process is considered to be a hindrance to sensitivity for the ICP methods (95). Occurrence of isobaric interferences hampers the accurate determination of certain elements (96). Another disadvantage is that ICP-MS is not available for every laboratory.

Numerous voltammetric procedures have been proposed for determination of traces of Cr (VI) (97, 98). The advantage of these procedures is a low detection limit and low cost of the apparatus used. The disadvantage of most of the procedures is the interference from surface active compounds present in natural samples (98). Although, a number of voltammetric procedures for chromium determination with low detection limit have been developed (99, 100), only some of them can be used for Cr (VI) determination in the presence of Cr (III) (101, 102).

1.6 Chemiluminescence Detection

Direct determination of chromium species demands very sensitive detectors. A detector system being sufficiently sensitive is inductively coupled plasma mass spectrometry which has been used in the determination of chromium species in aquatic samples. However, such an instrument is not available in most laboratories. As an alternative, the sensitive and more practical chemiluminescence detection can be used (103). Quantitative analysis based on chemiluminescence is very attractive not only because of its potentially high sensitivity and wide dynamic range but also because the instrumentation required is very simple (104).

1.6.1 Chemiluminescence (CL)

Light is energy transmitted by photons. If solids, liquids or gases are to emit light they require a source of energy. This energy can be

obtained either externally, for example by absorbing heat through conduction or radiation, or internally via physical or chemical changes such as nuclear transformations, electronic transitions or chemical reactions (105).

If a reaction is to be chemiluminescent, molecules must not only be generated in electronically excited states but also these excited molecules must be capable of either emitting photons directly, or transferring their energy to molecules which can. A chemiluminescent reaction therefore has three essential features (105).

- There must be sufficient energy generated by the reaction for the formation of the electronically excited state. In other words, the reaction must be exothermic.
- There must be a pathway by which this energy can be channeled to form an electronically excited state. If all the chemical energy is lost as heat, e.g. via vibrational and rotational energy, the reaction will not be chemiluminescent.
- The excited product must be capable of losing its energy as a photon, or be capable of energy transfer to a fluor. If all of the energy is lost or transferred via non-radiative processes, then the reaction will not be chemiluminescent (105).

An analytical chemiluminescent signal is produced by a chemical reaction and requires no light source for excitation (as in the more common fluorescence and phosphorescence). Its emission appears out of an essentially black background, and therefore the only background signal is that of the photomultiplier tube's dark current (106).

1.6.2 Chromium and CL

The CL technique is based on the light emission catalyzed by Cr (III) in the luminol-hydrogen peroxide reaction in basic aqueous solution. Luminol (5-amino-phthalhydrazide, otherwise known as 5-amino-2,3-dihydro-1,4-phthalazine dione) chemiluminescence was first published by Albrecht in 1928, although it had been synthesized in 1853. It has been one of the most well studied compounds and has long been known to produce light under certain reaction conditions.

Until the recent development of the chemistry used in "light sticks", luminol was one of the most impressive examples of chemiluminescence (chemically produced light). The reaction works by reaction between luminol, base, and hydrogen peroxide to produce an intermediate molecule that is very reactive. Instead of exploding to release energy as heat and gases (like some reactive molecules), the luminol-derived intermediate releases its excess energy as light energy (107) as shown in Figure 1.1.

Luminol is oxidized to 3-aminophthalate. The emission is blue, centered about 425 nm and is from the excited state 3-aminophthalate. Since the intensity of light is proportional to the concentration of free metal ion present in the sample, the technique is useful in the determination of speciation of chromium (108).

Detection of hexavalent chromium species, which is not chemiluminescence active is done after reduced to Cr^{+3} using sulphite (109).

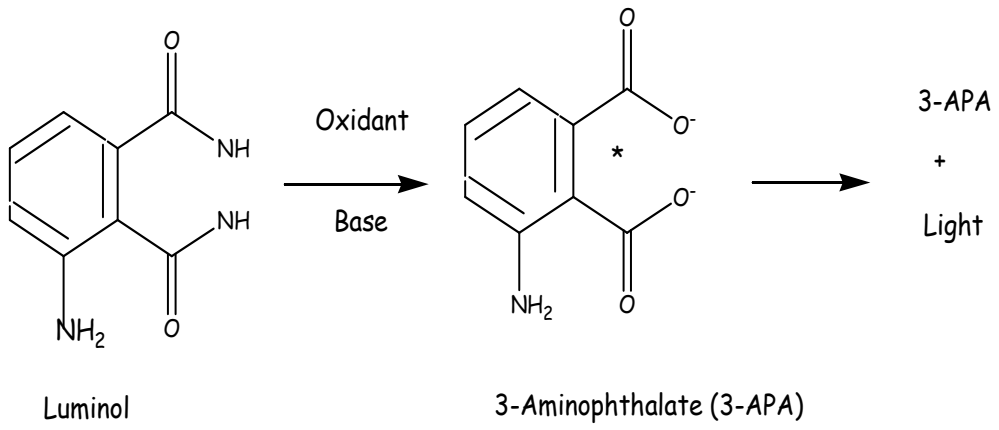

Figure 1.1 Reaction between luminol, base, and hydrogen-peroxide

1.7 Flow Injection (FI)

Historically, the first commercially available flow injection analysis system used air-segmented flow and was based on ideas proposed by Skeggs (110). Ruzicka and Hansen (111, 112) proposed the term "flow injection analysis" to describe a procedure in which the on-line formation of a derivative for a spectrophotometric monitoring was made by injecting a discrete volume of a sample into a continuously flowing carrier stream containing the reagent. All the parameters, such as sample injection volume, speed and time, carrier volume and speed, are controlled by a computer program.

There are detailed reviews of FI methods for chromium speciation, which report that LODs for Cr (III) are between 0.02 to 55 ng/mL, with a majority above 0.50 ng/mL, while LODs for Cr (VI) range from 0.02 to 20 ng/mL, with a majority above 1.0 ng/mL (113-115).

1.7.1 FAAS and FI

Flame AAS method is one of the most extensively used technique for chromium. However, FAAS technique needs a preconcentration step before determination since it is not sensitive enough to measure chromium content of a natural water samples. By fuel-rich air-acetylene flame sensitivities around 100-150 μg/L was reached. This technique is often used after speciation of chromium species so measurement in FAAS is made after applying a column which is used to separate and preconcentrate both chromium species (116).

1.7.2 FI and CL

CL reactions generate transient emissions and as a result it is necessary to execute all measurements under precisely defined and reproducibly maintained conditions so that all samples are treated physically and chemically in exactly the same manner. For these reasons CL leads itself to FI where all experimental parameters can be rigidly controlled and is reproducibly maintained. The noise and signal to noise ratio are primarily dependent on the composition and flow rate of the luminol-hydrogen peroxide reagent. Also the stability of reagents are important for practical use (104).

1.7.3 FI, CL and Flow-Cell

In order to measure the transient emission produced by CL reactions, the emitting species should ideally be in the observation field of the detector while the emission is occurring. As emission varies with time, the amount of light detected will correspond to that portion of the emission profile which occurs during the time interval spent in the detection cell. In a typical FI-CL manifold, the flow cell is placed in front of the detector. Various studies of chemiluminescence flow cell and flow system designs aim to minimize the loss of emission while the sample is on the way between the point where the reaction is initiated and the detector. Such a loss becomes significant for fast reactions where CL is generated within a short time span and the detection must occur immediately after reagent mixing (104).

The design of a flow cell for optical measurements in flowing liquid streams involves a compromise between flow characteristics of the cell and the detector optics. The desirable fluid flow properties of a flow cell are characterized by low or controlled dispersion, low dead volume and a well swept transition from one flow cross-section to another. Flow characteristics must also ensure that the cell is thoroughly rinsed between two analyses and it should be chemically inert, since many CL reactions are sensitive to trace concentrations of metal ions (104). Quantitative analysis based on chemiluminescence is very attractive not only because of its potentially high sensitivity and wide dynamic range but also because the instrumentation required is very simple (104).

1.8 Organically Modified Silicate Materials (ORMOSIL)

Sol gel process provides a convenient method for the production of organically modified surfaces by incorporating alkoxysilane monomers that contain desirable functional groups in the starting polymerization mixture (e.g. amino-, vinyl-, epoxy-, mercapto-). Organosilicon precursors ($R_{4-x}Si(OR')_x$, x=1-3), can be hydrolyzed and condensed with silicon alkoxides (117).

The blending of inorganic precursors (e.g. TMOS) with organoalkoxysilanes can lead to materials with properties better than those prepared alone. These materials termed ORMOSIL (organically modified silicates), (organic- inorganic hybrid materials) can be prepared by mixing organosilicon precursors of the general formula ($R_{4-x}Si(OR')_x$, where R represents the desirable reagent or functional group and x is 1-3, with TMOS, or alternatively alone. Specific functional groups that have been used include CH_3 , C_2H_5, C_6H_5, $(CH_2)_3NH_2$, $(CH_2)_3SH$ (117).

The organic modifier can be covalently bound to the silica-oxygen network and the organic component is physically embedded into the inorganic network or where organic monomers or polymers are introduced into the inorganic networks (117).

1.9 The Aim of the Work

Speciation and preconcentration of trace metals emerged as an important issue since different oxidation states of metals have different effects in the life of living organisms.

- A rapid, selective and sensitive method was designed for on-line preconcentration and speciation of chromium species in natural water samples by FAA and CL spectrophotometric techniques.

- The performance of amino silica-gel, for the solid phase extraction of chromium species is investigated.

- A chemiluminescence method based on the catalytic effect of chromium on the oxidation of luminol by hydrogen peroxide is investigated and parameters effecting the CL- detection of chromium species are optimized.

- Interferences were examined in terms of their effects both in the preconcentration and determination of chromium species.

- A home- made computer controlled flow injection analyses (FIA) system was optimized for the preconcentration and determination of chromium species utilizing FAAS and CL detection techniques.

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and Reagents

i) Cr (III) stock solution (1000 mg/L): Prepared by dissolving 0.512 g $CrCl_3.6H_2O$ in 100 mL of 0.5 M HCl.

ii) Cr (VI) stock solution (1000 mg/L): Prepared by dissolving 0.374 g K_2CrO_4 with 100 mL deionized water.

iii) 3-mercaptopropyl trimethoxy-silane: Alfa Products.

iv) γ-aminopropyl trimethoxy-silane: Alfa Products.

v) TEOS (tetraethoxysilane): 98%, Aldrich, d: 0.934, K_p: 168°, F.G: 208.33.

vi) Hydrochloric acid, Merck extra pure (35%), density: 1.19 g/mL.

vii) Ethanol, Aldrich, HPLC grade.

viii) Luminol, Aldrich Chem.Company.

ix) H_2O_2, E. Merck A.G.

All other reagents were of analytical grade. Deionized water obtained from an Elga Water Purification System was used for sample and standard preparations. Volumetric flasks, pipettes, beakers and plastic

ware were cleaned by soaking them in dilute (1+9) nitric acid at least 24 hours. Then, all glassware and plastic-ware were washed with distilled water and dried on coarse filter papers.

2.2 Apparatus

In this study two different measurement techniques were used. One is Flame Atomic Absorption Spectrophotometer (FAAS) and the other one is Chemiluminescence (CL) spectrophotometer.

PU 9200 FAAS was used for chromium measurements at 357.9 nm resonance line of chromium with a band-pass of 0.5 nm. The results in FAAS were printed with an Epson FX-850 printer connected to spectrophotometer. Fuel-rich air-acetylene flame with a 50 mm burner slot was used for the atomization. Instrumental parameters for chromium determination by FAAS are summarized in Table 2.1.

Table 2.1 Instrumental Parameters Used for the Determination of Chromium in FAAS

Light Source	Chromium Hollow Cathode Lamp
Wavelength	357.9 nm
Band Pass	0.5 nm
Lamp Current	10 Ma
Flame	Fuel-rich Air-acetylene
Burner Slot	50 mm

CL measurements were taken by Perkin Elmer LS50B Luminescence Spectrometer. Emission monochromator was set at 425 nm and the slits were positioned to 20 nm for photomultiplier tube to see all the light coming from the reaction cell. A Perkin-Elmer Stirred Cell Holder is used for mixing of solutions in the cell.

A new home-made computer controlled Flow Injection System (FIS) developed in our laboratory was used. The system consists of two syringe pumps, three 5-way distribution valves, tubings for connection, ferrules, nuts, unions, computer to control the system and electronic connections between the system and computer program.

2.3 Preparation of Amino Silica-gel and Mercapto Silica-gel Resins

A 5.0 g of silica gel (60-100 mesh size) were washed in 100 mL of 0.01 M acetic acid under vacuum for ten minutes. The contents were then filtered and silica-gel particles were transferred to 250 mL round bottom flask containing 25 mL of toluene. Three mL of γ-aminopropyl trimethoxy silane (amino silane) or 3-mercaptopropyl 3-methoxy silane (mercapto silane) were added dropwise over a period of 20 minutes by vigorous magnetic stirring (dispersal of the amino silane were intended in order to prevent self polymerization). About 0.15 mL of concentrated acetic acid was added dropwise such that a 0.1 M solution was obtained in toluene. The mixture was stirred overnight. Then 0.15 mL of concentrated acetic acid was added dropwise and the mixture was refluxed at

approximately 60 °C for two hours by magnetic stirring. Finally, sample was filtered using vacuum pump and washed with toluene before being left overnight in an oven at 80 °C (118).

2.4 Preparation of Amino Sol-Gel Resin

5 mL TEOS, 5.4 mL ethanol and 1.6 mL 1 M HCl were mixed immediately and the mixture was stirred for 30 minutes. A 500 µL aliquot of amino silane was diluted to 5 mL by 1 M HCl and added to the above solution dropwise. The mixture was stirred for one hour.

Then, the solution was allowed to rest at room temperature for 3 days in closed containers and dried to constant weight at 60 °C for approximately one week. After drying, the sol-gel monoliths obtained were crushed in a porcelain cup and sieved. The fractions of various particle sizes were collected for further experiments (119).

2.5 Chromium Uptake

2.5.1 Evaluation of Various Resins for the Sorption of Chromium

Different types of amphoteric oxides, such as alumina and Titania, silica-gel as acidic oxide, amino silica-gels and amino sol-gel (ORMOSIL) were tried to retain both chromium species (Cr (III) and Cr (VI) ions). Relatedly, 10 mL of 5 mg/L Cr (III) and Cr (VI)

solutions were prepared at different pH values (Table 2.2.). They were shaken with 0.1 g of the specified resins for one hour then filtered. The chromium content of the effluents were determined by FAAS.

Table 2.2 Compositions of Buffers

Reagents	Concentration (M)	pH
HCl	0.2 M	1.00
NaCOOH HCl	0.1 M 0.2 M	2.00
NaCOOH HCl	0.1 M 0.2 M	3.00
NaCOOH HCl	0.1 M 0.2 M	4.00
KHP NaOH	0.1 M 0.2 M	5.00
KHP NaOH	0.1 M 0.2 M	6.00
KH_2PO_4 NaOH	0.1 M 0.2 M	7.00
KH_2PO_4 NaOH	0.1 M 0.2 M	8.00
KH_2PO_4 NaOH	0.1 M 0.2 M	9.00
$NaHCO_3$ NaOH	0.1 M 0.2 M	10.00
$NaHCO_3$ NaOH	0.1 M 0.2 M	11.00
$NaHCO_3$ NaOH	0.1 M 0.2 M	12.00
Na_2HPO_4 NaOH	0.1 M 0.2 M	13.00

2.5.2 Optimization of the pH for the Sorption of Cr (III) and Cr (VI) with Amino Silica-gel, Mercapto silica-gel and Amino Sol-gel resins

Amino silica-gel, mercapto silica-gel and amino sol-gel resins were tried to retain both chromium species (Cr (III) and Cr (VI)). In order to see the effect of the pH on uptake behaviour of the resins, 10 mL of 0.1 mg/L Cr (III) and 10 mL of 0.15 mg/L Cr (VI) solutions were prepared separately from their stock solutions at different pH values (Table 2.2). The solutions were passed through micro-columns (Table 2.3) and the effluent solutions were analyzed for their chromium content by using FAAS.

2.6 Desorption of Cr (III) and Cr (VI) by Different Eluents

15 mL of 0.02 mg/L Cr (III) and 0.04 mg/L Cr (VI) solutions were prepared from their intermediate solutions at pH 8 and pH 2 (Table 2.2), respectively. Solutions were passed through the columns which are packed with amino silica-gel and amino sol-gel resins (Table 2.3). For the elution of both Cr (VI) and Cr (III), various concentrations of HCl (2 M, 4 M, 6 M) and 0.1 M NaOH were tried. Additionally for the elution of Cr (III), 0.1 % (w/v) $KMnO_4$ in 4 M HCl was used. In each case eluent volume was kept as 1 mL. Chromium content of effluents were determined by FAAS.

Table 2.3 Specifications of Different Columns.

	Mercapto Silica-gel	Amino Silica-gel	Amino Sol-gel
Particle size	100-150 µm	100-150 µm	100-150 µm
Weight	0.04 g	0.04 g	0.08 g
Column length	4 cm	4 cm	6 cm

2.7 Sorption Rates of Cr (III) and Cr (VI) ions

Different flow rates were examined in order to see the sorption rate of the amino silica-gel and amino sol-gel resins. Specifications of the columns are shown in Table 2.3. For Cr (III) studies each column was preconditioned with a pH 8 buffer, whereas for Cr (VI) studies, each column was preconditioned with a pH 2 buffer (Table 2.2).

In order to determine sorption rate of the resins for Cr(III), 15 mL portions of 0.02 mg/L Cr (III) solution were passed through the conditioned columns at different flow rates (0.5-5 mL/min). The retained species were eluted with 1 mL of 0.1 % (w/v) $KMnO_4$ in 4 M HCl at a flow rate of 0.5 mL/min. Similarly, for the calculation of sorption rate for Cr (VI), 15 mL portions of 0.04 mg/L Cr (VI) solution were passed through the columns at different flow rates (0.5-5 mL/min). The retained species were eluted with 1 mL of 6 M HCl at 0.5 mL/min. Chromium content of eluates were measured with FAAS.

2.8 The Reusability of Amino Silica-Gel for the Extraction of Cr (III) and Cr (VI) ions

It is expected that eluents used in this study are also functioning as regenerating solvents for the columns (Table 2.3). In order to examine the re-usability of the same column for the preconcentration of Cr (III) and Cr (VI) ions the following procedures were applied: For Cr (III), each column was preconditioned with a pH 8 (Table 2.3) buffer and then 15 mL of 0.02 mg/L Cr (III) solution was passed through the column at a flow rate of 1 mL/min. The retained species were eluted with 1 mL of 0.1 % (w/v) $KMnO_4$ in 4 M HCl at a flow rate of 0.5 mL/min. The same protocol, including the preconditioning step, was repeated 8 times with the same column. For Cr (VI), each column was preconditioned with a pH 2 buffer (Table 2.3) and then 15 mL of 0.04 mg/L Cr (VI) solution was passed through the column at a flow rate of 1 mL/min. The retained species were eluted with 1 mL of 6 M HCl at 0.5 mL/min. Again, the same protocol, including the preconditioning step, was repeated 8 times by using the same column. The chromium content of the eluates for each run were measured with FAAS.

2.9 Separation of Cr (III) and Cr (VI) from the synthetic mixtures

It was expected that at pH 2 (Table 2.2), Cr (VI) ions, whereas at pH 8, Cr (III) were selectively retained on the amino silica-gel. Therefore solutions having three different concentration ratios of Cr (III) to Cr

(VI) at two different pH values (pH 2 and pH 8) were prepared. The volume of the solutions was kept as 20mL. Amino silica-gel column (Table 2.3) was used for separation. Each 20 mL portion was passed through a separate column. Cr (III) was eluted with 1 mL of 0.1 % (w/v) $KMnO_4$ in 4 M HCl. Cr (VI) was eluted with 1 mL of 6 M HCl. The chromium content of the eluates were determined with FAAS.

2.10 Effect of Possible Interferences in the Recovery of Chromium Species

The effect of macro amounts of diverse metal ions like alkali, alkaline earth, some transition elements and anions potential to interfere on the performance of separation (using amino silica-gel column) and determination of chromium species (using FAAS) was tested. Therefore 10 mL of 0.04 mg/L Cr (III) solution containing possible interfering ions at different concentrations was passed through an amino silica-gel column (Table 2.3). The retained species were eluted with 1 mL of 0.1 % (w/v) $KMnO_4$ in 4 M HCl. Similarly, 10 mL of 0.08 mg/L Cr (VI) solution containing possible interfence ions at different concentrations were passed through an amino silica-gel column (Table 2.3). The retained species were eluted with 1 mL of 6 M HCl. The chromium content of the eluates were measured with FAAS.

2.11 Chemiluminescence Detection of Chromium

Light emission from CL system is proportional to the luminol, hydrogen-peroxide, and catalyst concentration. The effect of each parameter was studied by changing one at a time. Cr (III) ion behaves as a catalyst. Thus its concentration is determined directly. However Cr (VI) ion concentration can be measured only after reduction to Cr (III) form by sodium sulfite.

2.11.1 Effect of Luminol Concentration

0.01 M luminol prepared in 1 M KOH and 1 % H_2O_2 was used as stock solution throughout CL studies. Mixtures containing various concentrations of luminol and 0.06 % H_2O_2, as shown in Table 2.4, were prepared in a carbonate buffer at pH: 12 (Table 2.2). 2.1 mL of each mixture were placed in the cell compartment which was shielded from ambient light by using a black cartoon box. Chemiluminescence was measured immediately after the injection of 0.9 mL of 65 µg/L Cr (III) standard solution into the cell through a small hole.

Table 2.4. Mixtures of Different Conc. of Luminol and Same Conc. of H_2O_2

	Luminol Concentration (M)	H_2O_2 (%)
1st mixture	0.0010	0.06
2nd mixture	0.0020	0.06
3rd mixture	0.0030	0.06
4th mixture	0.0040	0.06

2.11.2 Effect of H_2O_2 Concentration

Mixtures containing various concentrations (in %) of H_2O_2 and 0.0035 M luminol, as shown in Table 2.5, were prepared in a carbonate buffer at pH=12 (Table 2.2). 2.1 mL of each mixture was placed in the cell compartment which was shielded from ambient light by using a black cartoon box. Chemiluminescence was measured immediately after the injection of 0.9 mL, 65 µg/L Cr (III) standard solution into the cell through a small hole.

Table 2.5 Mixtures of Different Conc. of H_2O_2 and Same Conc. of Luminol

	Luminol concentration(M)	H_2O_2 (%)
1st mixture	0.0035	0.02
2nd mixture	0.0035	0.04
3rd mixture	0.0035	0.06
4th mixture	0.0035	0.08

2.11.3 Effect of pH on CL Signal

Mixtures containing 0.16 % H_2O_2 and 0.0035 M luminol at different pH values (Table 2.2) are shown in Table 2.6. 2.1 mL of each mixture were placed in the cell compartment which was shielded from ambient light by using a black cartoon box. Chemiluminescence was measured

immediately after the injection of 0.9 mL, 65 µg/L Cr (III) standard solution into the cell through a small hole.

Table 2.6 The pH of the Mixtures of Luminol-H_2O_2

	Luminol concentration(M)	H_2O_2 concentration (%)	pH
1st mixture	0.0035	0.16	9.00
2nd mixture	0.0035	0.16	10.00
3rd mixture	0.0035	0.16	11.00
5th mixture	0.0035	0.16	12.00
6th mixture	0.0035	0.16	13.00
7th mixture	0.0035	0.16	14.00

2.11.4 Change of CL Intensity with Cr (III) concentration

2.1 mL of solution containing 0.16 % H_2O_2, 0.0035 M luminol at pH 11 (Table 2.2) were placed in the cell compartment which was shielded from ambient light by using a black cartoon box. Chemiluminescence was measured immediately after the injection of 0.9 mL of different concentrations of Cr (III) standard solutions (1-600 µg/L) into the cell through a small hole.

2.11.5 Determination of Cr (VI) with CL Technique

0.01 M solution of Na_2SO_3 at pH=4 (Table 2.2) was used as a reducing agent. 2.1 mL of solution containing 0.16 % H_2O_2 , 0.0035 M

luminol at pH 11 (Table 2.2) were placed in the cell compartment which was shielded from ambient light by using a black cartoon box. Chemiluminescence was measured immediately after the injection of 0.9 mL, 30 µg/L Cr (VI) standard solution containing 0.3 mg/L of reducing agent into the cell through a small hole.

2.11.6 Detection Limit of FI-CL system

For the preconcentration studies, 25 mL of 0.2 µg/L Cr (III) solution at pH=8 were prepared. Cr (III) species were loaded to a micro-column doped with amino silica-gel (Table 2.3) with a flow rate of 0.5 mL/min. Then, the retained Cr (III) ions were eluted with 1 mL of 1 % $KMnO_4$ in 4 M HCl. Because Cr (III) ions were eluted as Cr (VI), excess amount of (20 µL of 400 mg/L) Na_2SO_3 was added to the eluate to reduce $KMnO_4$ and Cr (VI). 0.2 g NaOH was also added in order to make the medium basic.

1.0 mL of solution containing 0.16 % H_2O_2, 0.0035 M luminol at pH 11.16 (Table 2.2) was placed in the cell compartment which was shielded from ambient light by using a black cartoon box. Chemiluminescence was measured immediately after the injection of 0.5 mL eluent.

2.11.7 Effect of Interferences in CL Detection

Cobalt (II), copper (II), aluminium (III) and nickel (II) ions were selected as potential interferants. Their concentrations were adjusted to a thousand fold higher than that of Cr (III) concentration (10 µg/L).

The effect of each interfering ion was examined separately. 2.1 mL of solution containing 0.16 % H_2O_2, 0.0035 M luminol at pH 11.16 (Table 2.2) were placed in the cell compartment which was shielded from ambient light by using a black cartoon box. Chemiluminescence was measured immediately after the injection of 0.9 mL of 10 µg/L Cr (III) standard solution containing 50 mg/L of EDTA and 10 mg/L of an interfering ion into the cell through a small hole.

CHAPTER 3

RESULTS and DISCUSSION

3.1 Preparation of Amino Silica-gel, Mercapto Silica-gel and Amino Sol-gel Resins

Silica gel was chosen as a substrate due to its low price, high surface area and low level of impurities.

Silica gel has hydroxyl functional groups. Their chemical behaviour are different than the amino groups in amino silane or mercapto groups in mercapto silane which are used to modify the surface of the silica gel by replacing already existing hydroxyl groups.

In the preparation of the amino silica-gel or mercapto silica-gel resin, the procedure which was developed earlier was used (118). Dilute acetic acid was used to wash silica gel in order to wet the pores of it. Addition of γ-aminopropyl trimethoxy silane (amino silane) or 3-methoxysilane (mercapto silane) was so slow for each droplet in order

to distribute itself among solvent molecules. Therefore, self-polymerization of amino silane was avoided.

Sol gel process provides a convenient method for the production of organically modified surfaces by incorporating alkoxysilane monomers that contain desirable functional groups in the starting polymerization mixture. In our laboratory sol-gel containing amino functional group was prepared. Amino groups are reactive toward many metals, besides they show selectivity to the oxidation state of the elements, and generally lower oxidation states are readily adsorbed (116,119).

3.2 Chromium Speciation and Preconcentration

During the past few years, increasing attention has been paid to chemical speciation studies. The main reason for this interest is the dependence of the toxicity on the chemical form in which an element appears. Cr (III) is considered to be an essential element, while Cr (VI) is known to be toxic and carcinogenic. Thus, determination of total chromium does not give an idea about possible toxicity.

The low inherent levels of chromium present in most samples (at low μg / L level) demands sensitive analytical methods which are able to distinguish between Cr (III) and Cr (VI).

In our studies speciation of chromium involves a separation and preconcentration by retainment of the chromium species on columns

and subsequent elution and determination by FAAS and CL techniques. Therefore, resins prepared in our group, mercapto silica-gel, amino silica-gel and amino sol-gel together with some other amphoteric and acidic metal oxides (alumina, titania, silica-gel) suggested for chromium speciation were examined for the sorption of both Cr (III) and Cr (VI).

3.3 Chromium Uptake

3.3.1 Evaluation of Various Resins for the Sorption of Chromium

A critical parameter in achieving the quantitative recovery of trace elements is pH. As a rule, sorption of cations on amphoteric oxides, such as alumina and titania, proceeds when the pH value of the solution is higher than the isoelectric point (IEP) of the oxide, whereas for anion sorption a pH value lower than IEP is required (120). At pH values higher than IEP, the oxide surface is covered by OH groups and is negatively charged. As a result, it becomes active toward cation sorption. In contrast, the positive surface charge at low pH is caused by sorbed protons and determines the ability of the particle for the sorption of anions.

The results on the effect of pH on the solid-phase extraction with mercapto silica-gel, amino silica-gel, amino sol-gel**,** and amphoteric and acidic metal oxides in batch conditions are presented in Table 3.1. It is seen that pH values higher than 7 is appropriate for sorption of Cr (III) on Al_2O_3, TiO_2, SiO_2, amino sol-gel and mercapto silica-gel. Whereas Cr (VI) sorption is attained at low pH values on Al_2O_3, TiO_2

and Amino Sol-gel. The abbreviation "x" refers to failure to retain the specified species and √ refers to success in the retention of the specified species and pH values.

Table 3.1 Performances of Various Resins for Solid-Phase Extraction of Cr (III) and Cr (VI) Species.

RESIN		pH				
		1	3	5	7	10
Al_2O_3	Cr^{3+}	x	X	x	√	√
	Cr^{6+}	√	√	x	x	x
TiO_2	Cr^{3+}	x	X	x	√	√
	Cr^{6+}	√	√	x	x	x
SiO_2	Cr^{3+}	x	x	√	√	√
	Cr^{6+}	x	x	x	x	x
Amino Sol-Gel	Cr^{3+}	x	x	√	√	√
	Cr^{6+}	√	√	x	x	x
Mercapto Sil-Gel	Cr^{3+}	x	x	√	√	√
	Cr^{6+}	x	x	x	x	x
Amino Sil-Gel	Cr^{3+}	x	x	√	√	√
	Cr^{6+}	√	√	x	x	x

3.3.2 Optimization of the pH for the Sorption of Cr (III) and Cr (VI) with Amino Silica-gel, Mercapto silica-gel and Amino Sol-gel resins

According to Table 3.2 amino silica-gel and amino sol-gel, represent very similar sorption properties to amphoteric oxides. Their surfaces are probably positively charged at low pH values and negatively charged at high pH values. Hence they become selective sorbents for anions at acidic conditions and for cations at basic conditions. On the other hand Mercapto silica-gel shows specific selectivity to Cr(III) ions only but not for Cr(VI) at any pH value. This behavior can not be explained by the formation of surface charge at the pH values higher than the isoelectric point of the mercapto groups. In fact similar behavior was observed in the preconcentration of arsenic (121), antimony (118), selenium (122) and tellurium (123) ions by using mercapto silica-gel. It takes up their lower oxidation states only over a wide pH range with a high efficiency.

The effect of pH on the retention of chromium species were re-examined in detail for mercapto silica-gel, amino silica-gel and amino sol-gel adsorbents. The percent take-up values of the resins for Cr (III) and Cr (VI) at the specified pH values are shown in Table 3.2 and Table 3.3 respectively. The results are given as the average of three replicates. X refers to chromium species is not retained on the column.

Table 3.2 Percent uptake values of mercapto silica-gel, amino silica-gel and amino sol-gel resins for Cr (III) for various pH values.

pH	Amino Sil-gel	Mercapto Sil-gel	Amino Sol-gel
3	X	X	X
5	42±5	27±3	33±4
6	88±5	48±3	74±4
7	96±5	76±4	87±3
8	102±6	90±7	92±4
9	90±6	97±6	91±2

Table 3.3 Percent uptake values of mercapto silica-gel, amino silica-gel and amino sol-gel resins for Cr (VI) for various pH values.

pH	Amino Sil-gel	Mercapto Sil-gel	Amino Sol-gel
1	85±4	X	76±3
2	102±6	X	98±3
3	70±6	X	88±3
5	20±8	X	27±3
7	X	40±6	X
10	X	20±3	X

As can be seen from Tables 3.2 and 3.3 mercapto silica-gel, amino silica-gel and amino sol gel resins can be used in the speciation and

preconcentration of Cr (III) species at pH values above 7. However for the preconcentration and speciation of Cr (VI) ions only amino silica-gel and amino sol gel resins are appropriate. Besides the extraction performance of amino silica-gel is slightly better than that of amino sol-gel for both oxidation states of chromium.

Considering the advantage of concentrating Cr (III) and Cr (VI) separately at different pH values amino silica-gel resin was selected to be used in this study. The optimum pH values for the solid phase extraction were chosen as 8 and 2 for Cr (III) and Cr (VI) ions respectively.

Mercapto silica gel can also be used effectively in the chromium speciation: Cr (III) ions can be selectively removed from the sample solution using mercapto silica gel at pH 9 and Cr (VI) can be found by difference after Cr (III) and total chromium content are measured with FAAS. If preconcentration of Cr (VI) ions is required a reduction step should be added to the total procedure.

3.4 Desorption of Cr (III) and Cr (VI) by Different Eluents

As mentioned before pH is the determining factor for the selective extraction of chromium species on to the amino silica-gel. Thus for the elution of the chromium species from the column 0.1 M NaOH and various concentrations of HCl were decided to be used.

3.4.1 Desorption of Cr (III)

Percent recoveries of Cr (III) species with the specified eluents is presented in Table 3.4. X refers to chromium species is not eluted with the specified eluent. As can be seen from the table, Cr (III) species can not be removed from the column with 0.1 M NaOH solution which was anticipated. On the contrary to our expectation, increased HCl concentrations did not change the recovery of Cr (III) ions. Even at very acidic conditions (4M or 6M HCl) the recovery of Cr (III) was only 50 percent. However when an oxidizing solution is used as an eluent quantitative recovery was achieved. Thus 0.1 % (w/v) $KMnO_4$ in 4 M HCl was chosen as an appropriate eluent for desorption of Cr (III) species from amino silica gel column.

Similar behavior has been observed during the removal of arsenic (121), antimony (118), selenium (122) and tellurium (123) ions from the mercapto silica-gel column. In all of these studies an oxidizing agent was used as an eluent. This may indicate that extraction of Cr (III) species on to the amino silica gel column at basic medium is not a simple ion pair formation.

Table 3.4 Percent recovery values for Cr (III) with various eluents for three replicates.

Eluent	% Recovery
2 M HCl	48±3
4 M HCl	52±3
6 M HCl	50±3
0.1 % (w/v) $KMnO_4$ in 4 M HCl	102±4
0,1 M NaOH	***X***

3.4.2 Desorption of Cr (VI)

Based on the pH take-up studies, It was proposed that the amino group of amino silica-gel becomes protonated (NH_3^+) in acidic media and holds anionic form of chromium (CrO_4^{-2}) by charge neutralization. Table 3.5 presents percent recoveries for Cr (VI) species with the specified eluents.

As can be seen from the table, at basic conditions (0.1 M NaOH) complete recovery was obtained. However, when the concentration of HCl was increased the recovery of Cr (VI) ions was also increased. Complete desorption of Cr (VI) was achieved with either 6 M HCl or 0.1 M NaOH. These two observations are in agreement with the hypothesis given above. Probably at basic conditions surface charge becomes neutral and at high acid concentrations the chromate anion

prefers to form chromic acid. In both case chromate ions will be washed out from the surface.

Table 3.5 Percent recovery values for Cr (VI) with various eluents for three replicates.

Eluent	%Recovery Cr(VI)
2 M HCl	66±3
4 M HCl	74±5
6 M HCl	101±5
0.1 M NaOH	98±4

3.5 Sorption Rates of Cr (III) and Cr (VI) ions

The application of a small eluent volume contributes to obtaining a high preconcentration factor and increasing sample volume results in an increased preconcentration factor but it also increases the analysis time. Hence, rapid sorption and desorption of Cr (III) and Cr (VI) species allow the analysis of many samples in a brief period with FIA system.

Recovery studies were carried out at various duration of solid phase extraction in order to find the sorption rate of amino silica-gel and amino sol-gel resins. The results are depicted in Table 3.6. At the conditions specified in the experimental section (2.8) the best flow rate is 0.5 mL/ min for both oxidation states of chromium.

Table 3.6 Effect of Flow Rate on the Recovery of Cr (III) and Cr (VI)

	Cr(III)		Cr(VI)	
Flowrate (mL/min)	% R with A.sil	% R with A.sol	% R with A.sil	% R with A.Sol
0.5	102±4	69±1	100±3	98±1
1.3	92±4	65±4	86±5	86±5
3.2	79±6	49±4	77±4	73±2
4.8	79±5	48±5	77±5	68±2
5.6	75±3	27±4	73±3	62±4

3.6 The Reusability of Amino Silica-Gel for the Extraction of Cr (III) and Cr (VI) ions

Regeneration of the resins is very important in flow injection systems because changing columns is not practical, time-consuming and expensive.

In order to investigate the reusability of amino silica-gel for the extraction of chromium species recovery studies were performed using the same column for 8 successive measurements. Results are presented in Table 3.7 and Table 3.8. As can be seen from the tables there is no change in the recovery values at all after 8 successive measurements.

Table 3.7 Recoveries obtained for Cr (III) by using the same amino silica-gel resin successively

Number of Re-use	% Recovery with Amino Silica-gel
1	86±4
2	83±3
3	83±2
4	83±4
5	83±1
6	83±1
7	90±4
8	86±3

Table 3.8 Recoveries obtained for Cr (VI) by using the same amino silica-gel resin successively.

Number of Re-use	% Recovery Amino silica-gel
1	82±2
2	82±4
3	84±4
4	85±4
5	83±2
6	82±3
7	79±2
8	83±2

3.7 Separation of Cr (III) and Cr (VI) from the Synthetic Mixtures

In order to see the effect of each chromium species on the extraction efficiency of the other, synthetic mixtures having three different concentration ratios of Cr (III) to Cr (VI) were prepared and recovery experiments for Cr (III) and Cr (VI) were conducted. Compositions of the species in the mixtures, recovered amounts and % relative errors are given in Table 3.9. As can be seen percent relative error [(Amount taken-Amount recovered)/ Amount taken x 100] values are changing in between 1.8 to 8.2 %. The magnitude of errors could have been reduced if the recovery value of an ion when it was extracted from the mixture solution was compared with the recovery value of the same ion when it was extracted from its standard solution.

Table 3.9 Separation of Chromium Species in Synthetic Mixtures

Species	Amount taken (μg)	Amount recovered (μg)	% Error
Cr (III)	0.80	0.75	6.3
Cr (VI)	0.80	0.79	1.8
Cr (III)	0.80	0.73	8.2
Cr (VI)	0.080	0.074	7.5
Cr (III)	0.080	0.076	5.0
Cr (VI)	0.80	0.74	7.3

3.8 Effect of Possible Interferences in the Recovery of Chromium Species

One can expect that, during the analysis of natural water, the sorption sites free from trace metals are occupied by magnesium or calcium ions. The total amount of these sites is determined by the exchange capacity of the separation column. A higher exchange capacity of the separation column results in a larger remaining amount of alkaline earth metals. The low affinity of alkali and alkaline earth elements toward amino silica-gel results in a good rejection of these elements from the column and thus convenient operation with natural waters.

We investigated the effect of some major (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) and trace (Co^{2+} and Fe^{3+}) cations and some anions (PO_4^{3-}, SO_4^{2-}, Cl^{-}, HCO_3^{-}, AsO_2^{-}) present in natural waters on the preconcentration of Cr (III) and Cr (VI). The results for the effect of foreign ions on the recovery of chromium species are presented in Table 3.10.
As can be seen from the Table 3.10 no obvious interference is to be expected from the normal contaminant levels found in natural waters. However, even low concentration of Fe^{3+} noticeably suppress the solid phase extraction of Cr^{3+}. The effect of Fe^{3+} seems to be due to blocking of the sorption sites.

Cr (VI) has a typical anionic sorption characteristic. Recovery of Cr (VI) was not affected in the presence of major cations. However, again low concentration of Fe^{3+} suppress the solid phase extraction of Cr (VI).

Table 3.10 Effect of foreign ions

Ions	Concentration (μg/L)	% Recovery Cr (III)	% Recovery Cr (VI)
Na^+	2000	97±3	100±4
K^+	2000	101±2	101±1
Ca^{2+}	2000	97±2	98±1
Mg^{2+}	2000	98±4	97±2
Fe^{3+}	100	82±2	95±5
Co^{2+}	100	103±5	100±2
PO_4^{3-}	1000	99±3	87±3
SO_4^{2-}	1000	100±2	93±4
Cl^-	2000	100±3	97±4
HCO_3^-	1000	99±1	98±2
AsO_2^-	500	102±5	94±3

3.9 Chemiluminescence Detection of Chromium

Catalytic effect of Cr (III) species on the oxidation reaction by hydrogen-peroxide is the basis for the chemiluminescence measurements. Cr (VI) species were measured following their reduction by sodium sulfite.

The parameters affecting chemiluminescence (CL) signal are luminol and hydrogen peroxide concentration and the pH of the solution in the CL cell.

3.9.1 Effect of Luminol Concentration

Figure 3.1 show the change of chemiluminescence intensity with the concentration of luminol. 0.035 M luminol concentration which results in the highest chemiluminescence intensity was chosen as the optimum luminol concentration.

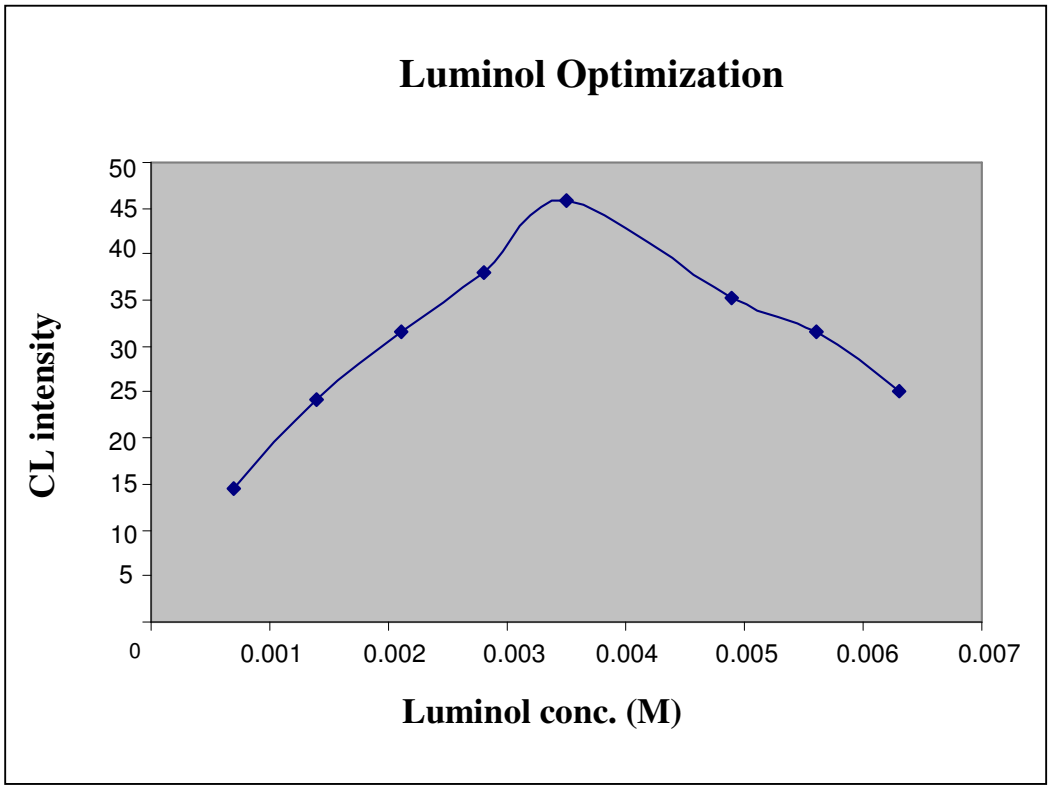

Figure 3.1 Change of CL Intensity with luminol concentration. Conc. of H_2O_2, Cr (III) and the pH of the medium are 0.06 %, 65 µg/L and 12, respectively.

The decrease in CL intensity at high concentration of luminol can be attributed to the formation of luminol-metal complex that reduces the availability of the metal ion for catalysis.

3.9.2 Effect of H_2O_2 Concentration

Light emission from luminol systems involving peroxide is proportional to the peroxide concentration. In Figure 3.2, it is seen that CL intensity increases up to 0.16 % peroxide concentration. After this point, a decrease is observed. With a higher hydrogen peroxide concentration, more noise is observed, probably as a consequence of decomposition of hydrogen peroxide.

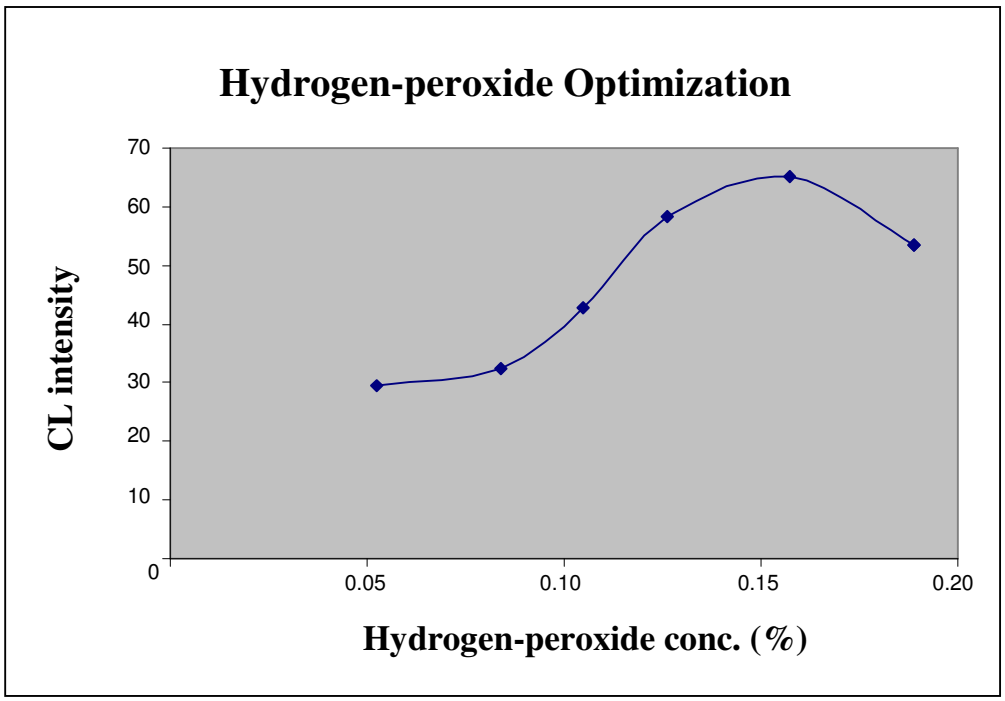

Figure 3.2 Change of CL Intensity with H_2O_2 concentration. Conc. of luminol, Cr (III) and the pH of the medium are 0.0035 M, 65 µg/L and 12, respectively.

3.9.3 Effect of pH on CL signal

The luminol-chemiluminescence reaction requires an alkaline pH, generally pH 9 to 11, depending upon the catalyst. For most catalysts the optimum pH for chemiluminescence is around 11. Figure 3.3 shows chemiluminescence intensity as a function of pH for Cr^{+3}. At pH 11 chemiluminescence intensity is 355.2 which is a maximum value and after this point it starts to decrease slowly. Thus, pH 11 was chosen as the optimum value for pH.

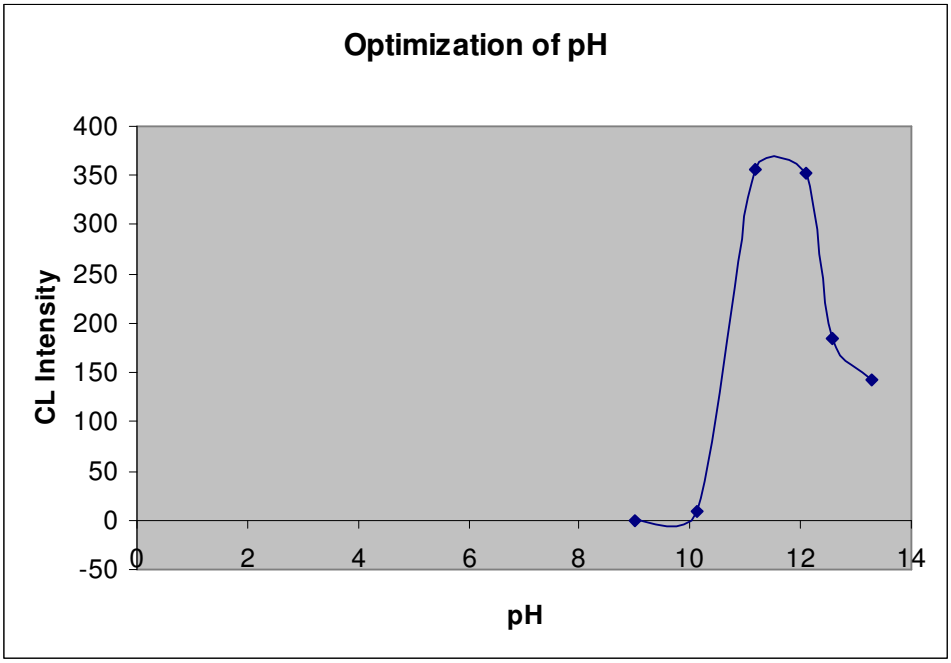

Figure 3.3 Change of Chemiluminescence Intensity with pH. Conc. of H_2O_2, Cr (III) and luminal are 0.16 %, 65 µg/L and 0.0035 M, respectively.

3.9.4 Change of CL Intensity with Cr (III) concentration

Oxidation of luminol by hydrogen peroxide takes place very slowly, so a catalyst is needed. Cr (III) has a catalytic effect in this reaction. Since the medium has to be basic, KOH solution is necessary for dilution of luminol. In the presence of base and peroxide most ions (Fe^{2+}, Cr^{3+}) react to produce forms that do not catalyze luminol chemiluminescence. CL reaction of luminol terminates when these species are converted to an inert form (Fe^{3+}, Cr^{6+}). These species are not true "catalysts" because they change during the course of the reaction.

There are a few "true" catalysts such as Ni^{2+}. Any Ni^{3+} formed during the course of the chemiluminescent reaction will react with water and go back to Ni^{2+}. The chemiluminescence detection of true catalysts can not be made since the reaction will proceed until all of the luminol-hydrogen peroxide in the medium is consumed.

In Figure 3.4., the catalytic effect of Cr (III) in the oxidation of luminol by hydrogen peroxide is depicted. As can be seen, up to 200 µg/L concentration of Cr (III), CL signal increases linearly. Afterwards the CL intensity does not change with concentration.

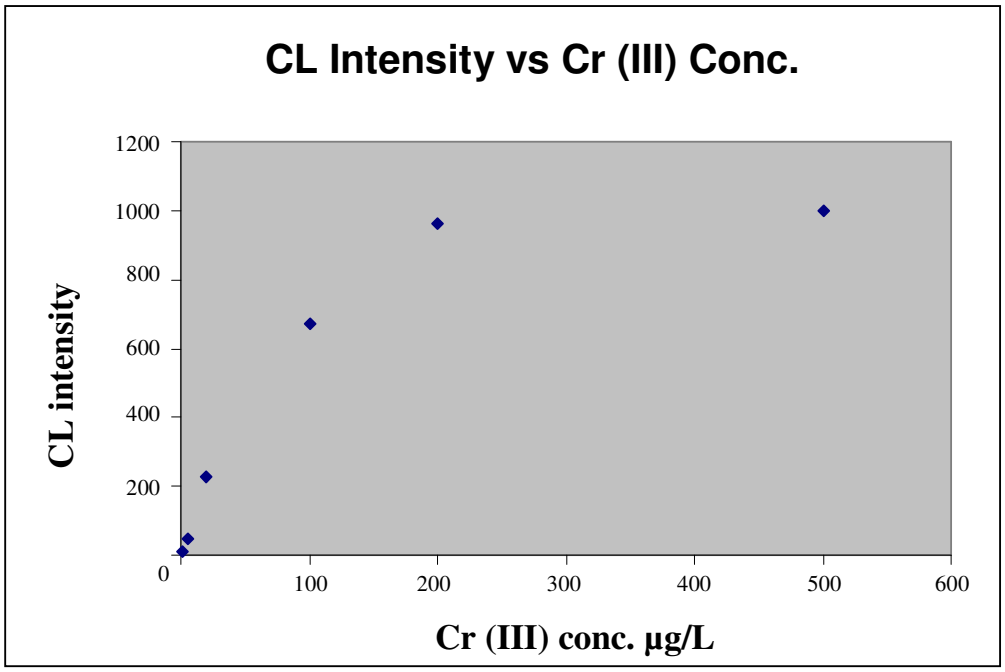

Figure 3.4 Change in chemiluminescence intensity with Cr (III) concentration. Conc. of H_2O_2, luminol and the pH of the medium are 0.16 %, 0.0035 M and 11, respectively.

3.9.5 Determination of Cr (VI) with CL Technique

Cr (VI) does not catalyze the oxidation of luminol by hydrogen peroxide. Hence it does not give any chemiluminescence signal. However after reducing to Cr (III), hexavalent chromium can also be determined using CL technique. In this study, sodium sulfite was used as the reducing agent. Table 3.11 shows the change in CL intensity due to the change in concentrations of Cr (III) and Cr (VI) ions.

Table 3.11 CL signals of Cr (III)and Cr (VI)

	Concentration of chromium species	CL Intensity
Cr (III)	10 µg/L	39
	100 µg/L	377
Cr (VI) (0.2 mL RA)	10 µg/L	31
(1.0 mL RA)	100 µg/L	404

RA: reducing agent

3.10 Detection Limit of FIA-CL system

The concentration of chromium in natural waters is around sub µg/L range. Hence preconcentration of 0.2 µg/L of Cr(III) was investigated using amino silane resin and CL technique. Recovery value was 84.5 ± 3.0 %.

3.11 Effect of Interferences in CL Detection

Any metal that can act as a catalyst can affect the CL signal. EDTA forms stable complexes with many metal ions. Metals complexed with EDTA do not interfere CL signal. Cr (III)-EDTA complex formation reaction is kinetically very slow i.e CL signal of Cr (III) is not influenced by the presence of EDTA. Hence, EDTA is frequently used in CL studies in order to reduce the interferences. Interference effects of metals in the presence of EDTA are presented in Table 3.12. It can be seen that, either Co^{2+} or Cu^{2+} changes CL signal. Therefore, both Co^{2+} and Cu^{2+} interfere and catalyze the reaction, raising the CL

signal. In the case of Ni^{2+} and Al^{3+} no change was observed in CL intensity.

Table 3.12 Effect of interferences

(Cr^{3+}-M) (1 µg/L-10 mg/L)	Chemiluminescence Intensity
Cr^{3+}	19
Cr^{3+}/Co^{2+}	>1000
Cr^{3+}/ Cu^{2+}	728
Cr^{3+}/ Ni^{2+}	21
Cr^{3+}/Al^{3+}	16

3.12 Computer controlled Flow Injection Analysis System (FIAS)

Home-made computer controlled FIA system, as shown in Figure 3.5, is consisted of two syringe-pumps, two five-port distribution valves, plastic tubing for the connections and micro columns packed with solid adsorbents. Syringes with various volumes (1-50 mL) can be accommodated to the system when needed. The optimization studies regarding the system are given below.

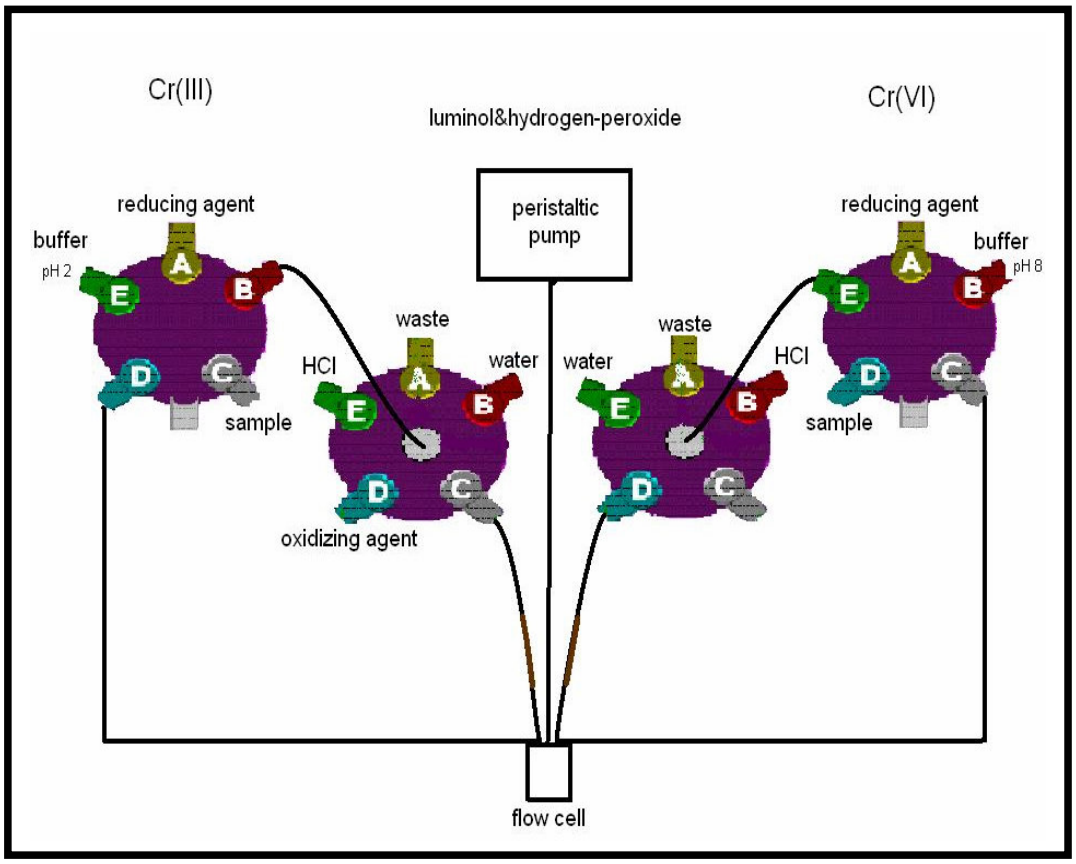

Figure 3.5 Flow Chart

3.12.1 Pump Syringe aspiration and dispense sample volume optimization

There are two types of aspiration commands and two types of dispense commands in the computer of FIA system. One is relative aspiration and absolute aspiration and the other is absolute dispense and relative dispense command. Aspiration and dispense volumes in a range 1 to10 mL have been tried for their validity in FIA system. The results given in Table 3.13 show that there are no significant differences between the volumes taken by both types of commands.

Table 3.13 Effect of Absolute and Relative Commands on Volume

	Aspiration (µL)	Dispense (µL)
RELATIVE	3000	2998
	3000	3004
	6000	5972
	6000	5976
ABSOLUTE	3000	2999
	3000	2998
	6000	5970
	6000	5957

3.12.2 Pump Syringe Aspiration and Dispense Speed Optimization

Different aspiration and dispense speeds were tried to see their effect on volume. The results given in Table 3.14 show that aspiration and dispense speeds also have no effect on volume. The results are very close to each other and the differences are not significant.

Table 3.14 Aspiration and dispense speed effect on volume

Aspiration speed (steps/sec)	Dispense speed (steps/sec)	Aspiration volume (μL)	Dispense volume (μL)
400	400	5000	4598
400	40	5000	4592
40	400	5000	4601
40	40	5000	4607

3.12.3 Stepwise Dispense

From the results in Table 3.15, it is clearly seen that there is always great difference between aspiration and dispense volumes. In order to see where the difference comes from, stepwise dispense experiments were done. It is seen in Table 3.15 and Table 3.16 that the difference or error always comes in the first milliliters of the dispense volume. Therefore, as a result in each experiment that will be done in the future for Cr species or others the first milliliters of the sample or eluting solutions should be discarded.

Table 3.15 Stepwise dispense of 5 mL by 1 mL increment

Aspiration volume(µL)	Dispense First mL	Dispense Second mL	Dispense Third mL	Dispense Fourth mL	Dispense Fifth mL
5000	712 µL	979 µL	983 µL	975 µL	998 µL
5000	683 µL	967 µL	1000 µL	985 µL	991 µL
5000	971 µL	1001 µL	987 µL	991 µL	957 µL

Table 3.16 Stepwise dispense of 2.5 mL by 0.5mL

Aspiration volume (µL)	Dispense 1-0.5 mL	Dispense 2-0.5 mL	Dispense 3-0.5 mL	Dispense 4-0.5 mL	Dispense 5-0.5 mL
2500	172 µL	452 µL	493 µL	485 µL	486 µL

CHAPTER 4

CONCLUSION

The low inherent levels of chromium present in most samples (at low μg / L level) demand sensitive analytical methods which are able to differentiate between Cr (III) and Cr (VI).

In our studies speciation of chromium involves a separation and preconcentration by retainment of the chromium species on columns and subsequent elution and determination by FAAS and CL techniques**.**

Considering the advantage of concentrating Cr (III) and Cr (VI) separately amino silica-gel resin was decided to be used. The optimum pH values for the solid phase extraction were chosen as 8 and 2 for Cr (III) and Cr (VI) ions respectively. The overall preconcentration efficiency, including both take-up and elution, is in the range of 84-101 %. Regeneration of the resins is very important in flow injection systems because changing columns is not practical, time-consuming and expensive. It was investigated that the same column can be used 8 times successively with the same efficiency.

A very sensitive chemiluminescence method was introduced and optimized for the determinations of chromium species.

By incorporating amino silica –gel colums in the CL measurements 25 fold enhancement was achieved in the sensitivity and the limit of quantitation was 0.2 μg/L of Cr (III). By this way the performance of the method for on-line determination of chromium in natural water was demonstrated.

A fully automated FI-CL system is designed that allows all necessary operations to be performed on-line. This system allows the pre-conditioning of micro-columns with different buffer solutions; adsorption of chromium species in micro-columns; washing these columns to remove interfering matrix components; elution of the species with minimum volume; transporting the species and chemiluminescence reagents to the cell; and, finally, cleaning of all pertinent conduits in the FIA-system in order to prevent carry-over between individual samples are done.

As a future work we can say that the accuracy of the method should be verified. And interference problems in CL measurement should be overcome.

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