Speciation of Chromium in a Chromium Enriched Yeast.

Marian Cullen B.Sc. (Hons).

Submitted for the award of Masters of Science.

Galway-Mayo Institute of Technology.

Supervisor: Dr. Joseph Scanlan.

Submitted to the National Council for Educational Awards, July 2000.

Abstract.

Speciation of Cr(VI) in Chromium Enriched Yeast.
by Marian Cullen.

The sample under investigation in this project is an experimental chromium enriched yeast used as a possible additive in animal foodstuff, which was produced by growing yeast in the presence of chromium (III) chloride. Chromium on its own in not biologically active but chromium in the form of chromium enriched yeast is biologically active. The objective of this project was to show the complete absence of chromium(VI) from the sample.

A literature survey describing previous work carried out on the speciation of Cr(VI) has been carried out. The principal methods of detection of Cr(VI) used in this project are Polarography, G.F.A.A. Spectroscopy, U.V. Spectroscopy and H.P.L.C. For each of the above methods a calibration curve was obtained and each method was applied to the yeast extract. The H.P.L.C. and U.V. spectroscopic method are specific for Cr(VI) but polarography and G.F.A.A. spectroscopy measure total chromium.

Tris-NaOH buffer has been investigated for the extraction of chromium(VI). Problems associated with air oxidation of Cr(III) in alkaline solution have identifed and procedures described for the suppression of air oxidation. Procedures are described for the application of the extraction procedure to the yeast extract and for the determination of Cr(VI) in the extract. Procedures are also described for the pre-concentration of Cr(VI) on a HPLC column and for the application to the yeast extract.

The rate of reduction of Cr(VI) by ascorbic acid is investigated and found to be first order with respect to ascorbic acid concentration. The reduction capacity of the yeast is also investigated and it was found that in acid solution the yeast will reduce Cr(VI) but in neutral or basic solution the reduction capacity is diminished. Conclusions regarding the objectives of the project are drawn and suggestions for further work are given.

Acknowledgements.

Firstly, I would like to thank Dr. Joe Scanlan, my project supervisor who provided advice and guidance throughout the duration of the project. I would like to thank the Department of Physical Sciences at the Galway-Mayo Institute of Technology for providing research facilities for the project. I would also like to thank the Technical Support Staff and the administrative staff of the college for their assistance. I would also like to thank the Industrial Liaison Office of G.M.I.T., and all the library staff, who helped in locating many books and papers. I would also like to thank my fellow M.Sc. and Ph.D. students, Steve, Lene, Siv, Siobhan, Ken, Michai and Steve for their moral support. I would like to thank Dr. Ronan Power of Alltech Biosciences who provided the yeast sample on which this project and thesis was based. And lastly, I would like to thank Enterprise Ireland and Alltech Bioseciences again for providing financial support for this project under the ARP scheme.

Contents.

1.0. Introduction.

1.1. Discovery and Sources of Chromite.

Although some three dozen chromium containing minerals are known, chromite is the major economically important source of chromium. Chromite is a dense, black mineral consisting primarily of iron (II) and chromium (III) oxides, with magnesium and aluminium oxides present in lesser amounts. The mineral is the principal source component of chromium ores. It has a unique crystalline structure, but its chemical composition will vary with the location of its source.

1.1.1. Discovery.

Chromite was discovered in the Ural mountains at the end of the 18th century, but the remoteness of the deposits limited supply and increased costs. In 1811, Isacc Tyson discovered chromite deposits north of Baltimore. The chromite from these deposits was exported to England for processing into chemicals for the textile industry. In 1845 Tyson began production of potassium bichromate at Baltimore to meet the demands of American textile mills and pigment manufacturers. Tysons mines were the worlds leading source of chromite during the second quarter of the 19th century. In 1848, rich chromite ore deposits were discovered in Istanbul and Turkey and these became the world's biggest source of chromite during the second half of the 19th century. During the first half of the 20th century, additional chromite deposits were found in Greece, Brazil, South Africa, Rhodesia (now Zambia and Zimbabwe), the Philippines, Albania and Madagascar. There has been no commercial mining of chromite in the US since 1961.

The total worldwide reserves of chromite are estimated to be 5.5 billion tonnes. Of this 1.5 billion tonnes are considered to be "economically exploitable" deposits, of which billions of tonnes are located in South Africa, and 1.3 billion tons in the Great

Dyke region of southern Zimbabwe. In terms of Zimbabwe's export earnings chromite is second only to gold. Estimates of the major chromite reserves in the "commercially exploitable" deposits are listed below. (Katz (1994)).

Table 1.1. Commercially Exploitable Chromite Reserves.

LOCATION	ESTIMATED RESERVES (tonnes)
South Africa	1.0×10^9
Zimbabwe	1.3×10^8
U.S.S.R.	1.2×10^8
India	7.9×10^7
Finland	5.0×10^7
Brazil	1.7×10^7
Philippines	1.2×10^7
Madagascar	1.0×10^7
Turkey	1.0×10^7

1.1.2. Sources.

Chromium compounds are components of the earth's crust, and their weathering is responsible for the natural ores of chromium in underground and surface waters, soils, "continental dust", and air. Their contributions to the total concentration of chromium in the environment are complemented by somewhat larger quantities of chromium released by human activities.

1.1.2.(a). Nonanthropogenic Sources of Chromium.

Chromium is the seventh most abundant element in the earth, but the crystal concentration of chromium is much lower than that of the mantel. (100μg/g v's

5000μg/g). The crust of the earth is highly heterogeneous, and the crystal rocks can be divided into the categories of igneous and sedimentary. The process of crystallisation of igneous rocks is complex. The process may begin with the solidification of the dense material olivine (Mg_2SiO_4) which sinks to the bottom of the melt and forms basalt. Insoluble iron salts solidify next, forming the minerals pyrrohotite (FeS), pyrite (FeS_2), magnetite (Fe_3O_4), chromite ($FeCr_2O_4$), and ilimenite ($FeTiO_3$). The bulk of the magma then solidifies to form a continuous series of rocks. Chromium compounds are released from these minerals during the physical, chemical and biological weathering process by which rocks are abraded and/or dissolved. Soils derived from serpentine are particularly rich in chromium. Once released, the chromium compounds can be transported as wind or water borne particulates.

Volcanism is an alternative to weathering for the release of chromium compounds. The chromium content of volcanic ash range from 400 to 500μg/g. Pacyna and Nriagu (1988) estimate the volcanic emissions of chromium to be 3,900 tonnes per year. They estimated the chromium emissions from windblown dust to be more than 10 time's greater, 50,000 tonnes per year.

1.1.2.(b). Anthropogenic Sources of Chromium.

The chromium content of fossil fuels are low, but the combustion of large quantities of coal (containing 5-50ppm chromium) and of oil (containing 0.1-1ppm chromium) are the major anthropogenic sources of environmental chromium in the United States. The ashes from the combustion of fossil fuels, which contain significant amounts of chromium, are disposed of by landfilling. Emissions from chromium chemical manufacturing facilities, from many cooling towers, and from steel mills are other major sources of airborne chromium.

Pacyna and Nriagu (1988) also reported that metallurgical processes are the major source of the anthropogenic chromium atmospheric emissions in Europe, specifically, European metallurgical processes contribute 15,420 tonnes of chromium per year to the atmosphere, and fossil fuel combustion in Europe contributes another 2.779 tonnes of chromium to the atmosphere each year. In the US during 1988, land disposal of chromium from the manufacturing and processing facilities alone was estimated to be 22,000 tonnes. Kilau and Shah (1984) reported an annual land disposal of 98,000 tonnes of chromium in the slags from stainless steel production, and Rai and Szelemecka (1990) estimated " a total of 17,000 tonnes of chromium contained in fly ash is deposited on the land surface in the US annually". When the contributions from these and those from atmospheric fallout, are added to the tonnage from manufacturing and processing, the annual antropogenic deposition of chromium into/onto the land is easily increased by at least two orders of magnitude.

On a global scale, anthropogenic chromium emission to the atmosphere amounts to 75,000 tonnes per year. (Katz (1994)).

1.1.3. Production of Ferrochromium and Chromium.

Ferrochromium for stainless steel and other alloy production is prepared by the reduction of chromite with carbon. High carbon ferrochromium was prepared by heating mixtures of chromium and iron oxides with powdered charcoal as early as 1821.

Chromium metal may be obtained by reducing chemically produced Cr(III) oxide (Cr_2O_3) with powdered aluminium. The reduction of Cr_2O_3 with silicon in the electric arc furnace has also been employed for the production of chromium metal, as has the electrolysis of chromic acid (CrO_3) containing small amounts of sulphuric acid.

1.1.4. Production of Chromium Chemicals.

Chromium chemicals became commercially available in the early 19th century with the discovery that chromium could be produced by roasting chromite with soda ash and lime. From the 1820's to the early 1940's, the high-lime process was used to produce chromates at the Rutherglen works near Glasgow.

Tyson's 1845 potassium bichromate plant at Baltimore used a similar process for the production of chromium chemicals.

In the years between the two world wars, the Mutual Chemical Company of America introduced rotary kiln roasting of chromite that had been ball-milled to less than 100 mesh size and mixed with soda ash and lime. From the turn of the century to the mid 1970's chromates were produced from chromite ore by three manufacturing facilities in Hudson county, New Jersey. The chromite was pulverised to 100 mesh size, mixed with soda ash and lime, and roasted at temperatures between 1100-1150^{o}C in rotary kilns to convert the insoluble trivalent chromium compounds in the ore to the more soluble sodium chromate. The sodium chromate was leached from the roast with water and converted to a variety of commercial chromium chemicals.

1.1.5. Uses of Chromium Compounds.

There is a wide range of industrial and commercial uses of trivalent and hexavalent chromium compounds. The inertness of the trivalent chromium oxides makes chromium compounds useful as corrosion inhibitors and as agents for anodising and plating metals. The colours of trivalent and hexavalent chromium compounds with appropriate solubility characteristics, makes them attractive as pigments. Other chromium compounds have been used for sensitising solutions for lithography and photoengraving. The oxidising properties of hexavalent chromium compounds have

found applications in the synthesis of organic dyestuffs, while other chromium compounds are used in the dying process. The tanning of leather is possible in hours rather than days because of the tendency of trivalent chromium to form complexes with basic oxygen and/or nitrogen atoms in proteins. Chromium compounds have also been used for pyrochemical purposes. i.e. fire works and some safety matches. Chromium and its compound are ubiquitous in modern society,

1.1.5.(a). Chrome Tanning of Leather.

Commercial development of chrome tanning of leather began at the end of the 19th century and the use of chrome tannage offers several advantages over the use of older vegetable tannins, in that the time required is comparatively short (hours) and the leathers produced by this process are more resistant to wear and heat than those produced by vegetable tannins. Chrome tanning produces a soft, flexible leather. (Katz (1994)).

1.2. Chromium in the Environment.

1.2.1. Chromium in Air.

Ehman et Al, (1987) identified hexavalent chromium in air borne particulates from Texas. Arar et Al (1992), cite 100% conversion of trivalent chromium to hexavalent chromium when sewage sludge conditioned with lime and ferric chloride are incinerated, and they determined hexavalent chromium in the airborne emissions from sewage sludge incinerators. Both Ehman et al and Arar and her associates found that hexavalent chromium was reduced during/after collection of the samples. Environmental transport of the chromium compounds emitted from coal-fired steam generators followed that of windblown dust.

1.2.2. Chromium in Water.

Trivalent chromium compounds are deposited into the sea as particulates from the atmosphere. The ultimate fate of these particulates is their incorporation into the seabed sediments. The behaviour of particulate bound chromium compounds transported to the sea by river water is similar to that of the chromium in particulates deposited in the sea from the atmosphere. Cutshall et al (1966), who measured chromium radioactivity in filtered Pacific Ocean water 525Km from the mouth of the Columbia River, proposed that "any Cr(III) in the ocean or in the river would associate with particles and be lost to sedimentation, while Cr(VI) would remain in solution." They concluded that the reduction of hexavalent chromium to trivalent chromium occurred "only very slowly if at all" and that "losses due to reduction to Cr(III) are small." Pettine et al (1991), have investigated oxidation kinetics of Cr(III) to Cr(VI) by hydrogen peroxide in an artificial seawater and in samples collected from the coastal waters of the Tyrrhenian Sea 80km south of Rome. They proposed a mechanism in which complexation with borate retarded the sedimentation and increased the oxidation of trivalent chromium. They estimated the half-life of trivalent chromium to be 45 days in surface seawater at a pH of 8 and at a temperature of 25°C.

1.2.3. Chromium in Soils.

The release of chromium from terrestrial sediments and soils is of concern both from fertility/phototoxicity standpoint and from considerations of groundwater contamination/public health. Gupta's (1984), triphasic soil system model, reproduced in figure 1.1 provides a convenient reference frame for the terrestrial transport and fate of chromium components.

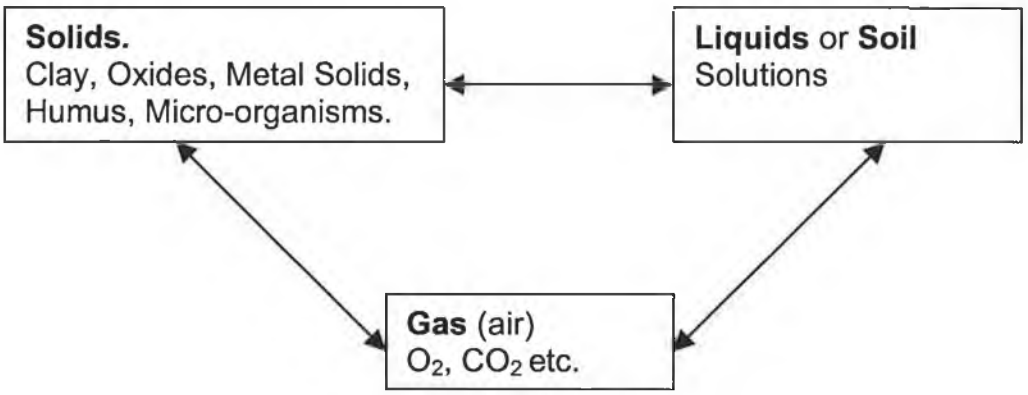


Figure 1.1 Triphasic Soil System.

The gas phase (air trapped in soil) is approximately 25% by volume of the soil system. The concentrations of metallic compounds with the possible exception of mercury vapour, are insignificant in the gas phase. The solid phase includes a variety of inorganic and organic materials that constitute the skeletal framework or matrix of soils. The liquid phase (soil solution) is approximately 25% by volume of the soil system. Clearly transport of chromium to and from the solid must take place via the soil solution. Factors such as pH (as well as pO_2 and pCO_2), solubility, cation (and anion) exchange capacity (CEC), and channel/pore size influence the transport of soil in soil system.

Bartlett and James (1979), demonstrated that trivalent chromium was oxidised to hexavalent chromium in fresh soil samples only; dried soil samples lost all capability of oxidising Cr(III) to Cr(VI). Oxides of manganese served as the electron link between the Cr(III)/Cr(VI) redox couple and atmospheric oxygen. The oxidation of trivalent chromium was controlled by the surface characteristics of these oxides and its transport to this surface. The oxidation of trivalent chromium was favoured by its speciation and mobility in the soil and by the age of the immobile manganese(IV) oxide surface and its freedom from absorbed, reduced organics and divalent

manganese. Organic acids were appropriately characterised as double-edged redox swords by Bartlett in that they participated in the oxidation-reduction of both redox couples. The reduction of hexavalent chromium by organic (humic, fulvic, gallic, oxalic, citric etc.) acids was slow. The half time for the reduction of hexavalent chromium by the organic material naturally present in soil was several weeks.

The processes that control the environmental chemistry of chromium have been identified by Rai et al (1989), as oxidation-reduction reactions, precipitation-dissolution reactions, and adsorption-desorption exchanges. Divalent iron, divalent sulphur and organic materials in the soil were among the potential reductants cited for hexavalent chromium, and manganese dioxide was given primary consideration for the oxidation of trivalent chromium to hexavalent chromium. The Eh-pH diagram (figure 1.2) shows the chromium species dominant under equilibrium conditions and their potentials for oxidation and/or reduction. The solubility of trivalent chromium was very low at the pH values typical for most soils. The influence of pH on solubility of trivalent chromium is shown in the pH solubility diagram. (figure 1.3). (Katz (1994)).

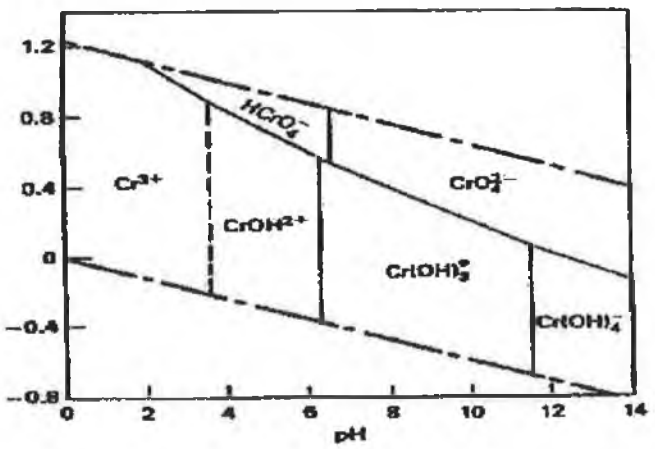


Figure 1.2. Eh-pH diagram for the various chromium species.

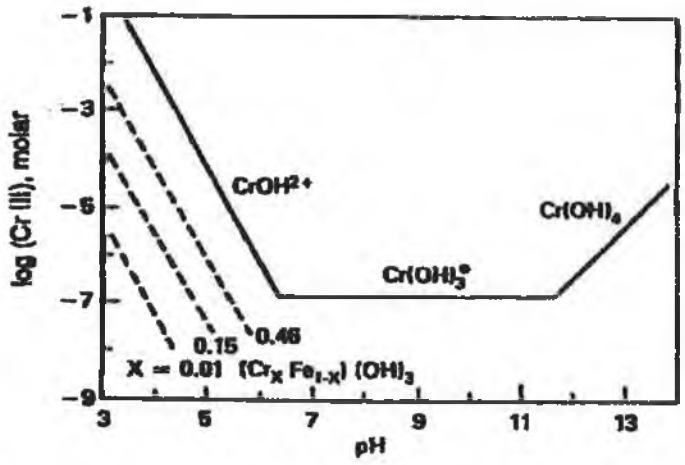


Figure 1.3. pH-Solubility diagram for various chromium species.

1.3. Chromium Uptake, Transportation, Distribution and Excretion.

1.3.1. Chromium in Foods.

In food consumed by humans and animals it is important in which form chromium is present. Biologically active chromium can be found in unrefined sugar syrup from beet and cane, in wheat germ, in black pepper and in beer yeast so therefore these products are useful to prevent chromium deficiency. Fruits contain very little chromium. But more than half of the dietary chromium is originated from other sources (i.e. preparation and cutlery) rather than the foodstuffs themselves. Stress, such as acute exercise, may lead to a reduction of chromium uptake. (Katz (1994)).

1.3.2. Chromium in Living Organisms.

The chromium content of seafish is 0.03-2mg/kg (dry weight). The muscles of mammals contain 0.002 to 0.8mg/kg and mammalian bones 0.1-30mg/kg. In food derived from animals chromium is biologically available in varying doses. Highest amounts of biologically active chromium are found in liver and cheese. Adult

humans contain about 5-20mg of chromium, particularly in the spleen and liver. The daily requirement of a human is 0.01-0.04mg of organically complexed chromium or about 0.1-0.03mg of chromium in an inorganic form. (Katz (1994)).

1.3.3. Chromium Uptake by Cells.

The oxidation state of chromium strongly influences the rate of chromium uptake. Cr(VI) can easily cross the cell membrane, where the phosphate-sulphate carrier also transports the chromate anions (figure 1.4). Cr(III) does not utilise any specific membrane carrier and its entrance into the cell is obtained by less efficient mechanisms, which is simple diffusion and in the case of animal cells, endocytosis. Cr(III) uptake depends on the nature of the ligand it is complexed with, to such an extent that in some instances cell membranes are particularly impermeable to Cr(III) complexes. Complexes with appropriate lipophilic ligands diffuse into cells with relative ease.

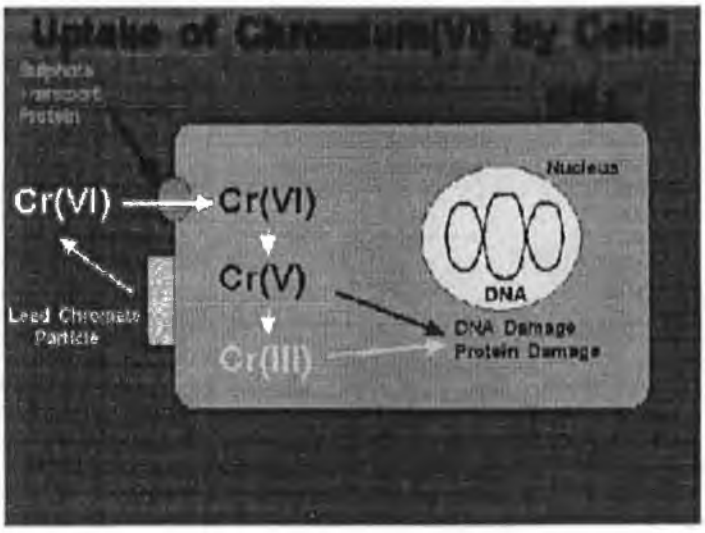


Figure 1.4. Uptake of Cr(VI) by Cells.

The efficient uptake of Cr(VI) by organisms and the manifold toxic effects of Cr(VI) inside the cell, make Cr(VI) contamination a serious environmental hazard in many industrial countries. Cr(VI) is rapidly reduced to Cr(III) inside the cell and its

biological activity depends on the process of its reduction and the subsequent trapping of Cr(III) in different cell compartments. Even in organisms exposed to Cr(VI) chromium is detectable in the reduced state. (Cohen (1993)).

1.3.4. Chromium Uptake within the Body.

The physiological exposure of humans and animals to chromium takes place in the gastro-intestinal (GI) tract, where chromium is introduced with the diet as Cr(III). Because there is a short time of contact of the chromium containing material with the GI epithelia, absorption is influenced by the nature of the Cr(III) complexes. Inorganic Cr(III) is poorly adsorbed, while Cr(III) linked to amino acids or other biomolecules is more readily taken up by diffusion across the plasma membranes. Such organic complexes–referred to as biological active chromium (BAC)-are the most efficient supply of chromium for humans and mammals. Trace amounts of absorbed chromium (below 1μg/day) are sufficient to maintain chromium balance in man. But because of the low percent absorption, the optimal dietary intake is in the range of 50-200μg/day. (Guthrie (1982), Langard (1982) and Offenbacher (1986)).

1.3.5. Chromium Transportation and Distribution within the Body.

Absorbed chromium is transported within the body by blood, Cr(III) is bound to plasma proteins, especially transferrin, and Cr(VI) is accumulated by the red blood cells (RBC) where it is reduced to Cr(III). Chromium transported by blood is distributed to tissues and organs, which have different retention capacity. The highest levels of chromium are found in liver, kidneys, spleen and lungs. The data on chromium distribution in the body derive from different sources, namely, non-exposed humans, occupationally exposed workers and experimental mammals. Each

set of data has its limitations and published values of chromium levels in the organs and tissues (eg. liver and blood) of control humans may vary within several orders of magnitude due to analytical problems and/or sample contamination before chromium measurements. (Langard (1982), Guthrie (1982) and Weber (1983)).

1.3.6. Chromium Excretion from the Body.

The main route of chromium excretion is through the kidneys with urine, also after exposure to Cr(III). Two phases can be detected in chromium excretion, a rapid one, corresponding to the clearance of chromium from the blood, and a slower phase representing the clearance from the tissues. Excretion with bile appears to be marginal.

1.4. Toxicity of Chromium.

1.4.1. Lethality of Chromium Compounds.

An oral dose of 2-5g of soluble hexavalent chromium can be fatal to an adult human. The toxic symptoms demonstrated by an adult human after ingestion of 5g of a soluble hexavalent chromium compound included gastrointestinal bleeding, massive fluid loss, and death from cardiovascular shock. Humans ingesting less than 2g of a hexavalent chromium compound have developed kidney and liver damage post-exposure.

The NIOSH Registry data on the acute oral toxicity of trivalent chromium compounds to the rat, listed LD_{50} values ranging from 1900 to 3300 mg/kg. By comparison the LD_{50} for sodium chromate was reported to be between 50 and 150 mg/kg. In general, the hexavalent chromium compounds appear to be 10-100 times more toxic than trivalent chromium compounds by the oral route of acute exposure. Some values for

the toxicity of trivalent and hexavalent chromium compounds are outlined in Table 1.2. In progressing from sodium chromate to calcium chromate to strontium chromate, both solubility and toxicity decrease. This trend may reflect the bioavailability of hexavalent chromium from these compounds. Similarly, the very low toxicity of Cr(III) acetate may reflect low availability of trivalent chromium from the stable acetato chromate (III) ion. (Katz (1994)).

Table 1.2.: Acute Oral Toxicity of Some Chromium Compounds to the Rat.

Compound	Name of Compound	LD_{50} / $mgKg^{-1}$	LD_{50} / $mmolKg^{-1}$
$CrCl_3.6H_2O$	Chromium (III) chloride	1870	7.0
$Cr(CH_3COO)_3.H_2O$	Chromium (III) acetate	11260	46
$Cr(NO_3)_3.9H_2O$	Chromium (III) nitrate	3250	8.1
CrO_3	Chromium(VI) oxide	80-114	0.8-1.1
$NaCrO_4$	Sodium chromate (VI)	52	0.32
$CaCrO_4$	Calcium chromate (VI)	327	1.7
$SrCrO_4$	Strontium chromate (VI)	3118	15
$Na_2Cr_2O_7$	Sodium dichromate (VI)	51	0.39
$K_2Cr_2O_7$	Potassium dichromate (VI)	57	0.39
$(NH_4)_2Cr_2O_7$	Ammonium dichromate (VI)	54	0.43

1.4.2. Effect of Chromium Compounds on the Kidneys.

Renal damage is the most frequently observed consequence of ingesting chromium compounds. Chromium is selectively accumulated in the proximal convoluted tubule. Exposure to high concentrations of chromium compounds cause acute tubular necrosis. The initial symptom is a marked reduction in urinary output, (200ml/day).

If death does not occur from renal failure or from the pulmonary or gastrointestinal toxicity of chromium, this oliguric phase is followed by a polyuric phase in which the output of urine exceeds 3L/day. Subsequently tubular regeneration and restriction of renal function result in recovery. (Katz (1994)).

1.4.3. Effect of Chromium Compounds on the Liver.

Acute oral chromium intoxication has been reported to cause damage to the human liver. (Toxicological Profile for Chromium 1991). Daily IP injections of rabbits with 2mg/kg of chromium either as chromium (III) nitrate or as potassium dichromate for several weeks produced congestion and dilation of the central veins and sinusoids, discrete foci of necrosis and haemorrhage in the parenchyma, nuclear pleomorphism, multinucleated cells in the lobules, and bile duct prilifieration. In rats treated for 60 days with 2mg/Kg of chromium by IP injection 3 times per week, the liver damage caused by sodium chromate was more severe than that caused by chromium (III) chloride. (Katz (1994)).

1.5. Carcinogenicity of Chromium.

Many of the earliest attempts to determine which species of chromium compounds were the causative agents for occupationally related cancers utilised inhalation or parenteral exposures with metallic chromium, chromate ore, or several commonly utilised chromium compounds. Although the majority of these studies yielded negative or equivocal results, several studies showed that Cr(VI) species were often carcinogenic from epidemiological studies, from animal experiments and from in-vitro genotoxic or mutagenic tests.

1.5.1.(a). Epidemiological Studies.

The greatest levels of exposures to Cr(VI) occur primarily during chromate production, welding processes, chrome pigment manufacture, chrome plating, and spray painting. Exposure to other valence forms of chromium occur primarily during mining ferrochromium and steel production, and during the cutting and grinding of chromium alloys. Epidemiological studies have played a key role in identifying some hexavalent chromium compounds as respiratory carcinogens. As early as 1890, Newman described an adenocarinoma of the inferior turbinate in the nose of a Scottish chromate pigment production worker. In 1935, Pfeil reported lung cancers among German workers who used dichromates to oxidise anthracene and its derivatives for the production of alizarin dyes. Alwens and Jonas (1938) later reported 20 cases of lung cancer among the workers at a chromate producing facility at Greisheim, near Frankfurt.

1.5.1.(b). Animal Experiments.

Oral exposure to chromium compounds does not result in enhanced tumour formation in test animals when compared with vehicle controls. Rats and mice provided with chromic acetate in their drinking water for life did not develop tumours at various body sites at greater rates than did controls. When Cr_2O_3 (1800 to 2850 mg/kg/day) was ingested along with other solids (i.e., baked in bread), there again was no enhanced incidence of tumours. Similar long-term feeding studies with Cr(VI) showed that while the total incidence of cancers did not vary from the controls, the development of forestomach carcinomas (as opposed to forestomach papillomas only) occurred only in rats fed K_2CrO_4 in their drinking water at 9 mg/kg/day for 900 days.

Although the corresponding linkage or route of exposure and sites of tumour formation are remarkably similar between humans and mice/rats, this susceptibility to tumour formation varies widely among the commonly used animal models. For example, mice and rats exposed to $ZnCrO_4$, $CaCrO_4$, or $Na_2Cr_2O_7$ atmospheres or intratracheal implants displayed a greater incidence of lung squamous metaplasias, subsequently followed by lung adenomas or adenocarcinomas, than did controls.

Direct inhalation and intratracheal, intrapleural, or intrabronchial instillation of Cr(VI) compounds are by far the most common routes of exposure, and tumour formation in animal models most often occur at these sites of deposition. The preponderance of data indicates that neither metallic nor trivalent chromium give rise to lung tumours. (Cohen, M. D. 1993).

1.5.1.(c). Genotoxicity.

A variety of short-term tests using different targets and/or end points have been employed to assess genotoxicity.(De Flora and Wetterhahn 1989). These assessments of genotoxicity were undertaken to compliment the epidemiological studies and the animal experiments used for establishing the carcinogenicity of chromium and its compounds. The results obtained from these tests show that the large majority of the positive results were obtained from hexavalent chromium compounds. Trivalent compounds, although more reactive than the hexavalent compounds with purified nucleic acids, did not induce genotoxic effects in the majority of the studies conducted with intact cells. With few exceptions, solutions of hexavalent chromium compounds were consistently positive in cellular systems. Almost 400 out of 450 tests showed that the soluble hexavalent chromium compounds were mutagenic in bacteria. In addition, the soluble hexavalent chromium compounds induced a broad range of

genetic effects in yeasts and insects. Soluble trivalent chromium compounds were inactive in cellular systems, but they produced a variety of effects in acellular or subcellular targets. The frequency of positive results with hexavalent chromium compounds was related to their solubilities, hence their bioavailabilities to the target cells. Although some trivalent chromium compounds appear to be capable of producing genetic effects when directly challenged with purified nucleic acids or with subcellular targets, their potential genotoxicity is lost in cellular systems. The results obtained from these genotoxic tests are summarised in Table 1.3. (Katz (1994)).

Table 1.3.: Results of Genotoxic Tests.

	Cr(VI) Compounds	Cr(III) Compounds
Positive Percentage	88.2	23.0
Negative Percentage	8.50	67.5
Total Tests	450	209

1.5.1.(d). Mutagenicity.

A common means of detecting mutations in micro-organisms is the selection for reversion in the strains that have a specific nutritional requirement different from that of the wild-type members of the species. The Ames test (Amdur et Al 1991) has received wide attention and utilisation as an assessment of mutagenicity and indicator of carcinogenicity. These tests employ several strains of *Salmonella typhimurium* that lack phosphoribosyl ATP synthetase, as the enzyme required for histidine synthesis. These strains, designated *S. typhimurin his (-)*, are unable to grow in histidine-deficient media unless reversion or back-mutation to the wild type takes place. The Ames test measures such reversions and thereby makes possible mutagenicity

assessments of many chemical compounds and mixtures of compounds. These assessments of mutagenicity have been interpreted as indicators of carcinogenicity. Using this Ames test only compounds containing hexavalent chromium demonstrated mutagenicity such as $CaCrO_4$, $SrCrO_4$ and $PbCrO_4$. None of the trivalent chromium compounds tested demonstrated mutagenicity. (Katz (1994)).

1.6. Essentiality of Chromium.

Chromium as an essential nutrient helps maintain normal metabolism of glucose, cholesterol and fat. Chromium deficiency leads to glucose intolerance. The criteria established for the essentiality of chromium are;

(i). The element is found in all healthy tissues of living organisms.

(ii). Its concentration is fairly constant from species to species.

(iii).Withdrawal of the element reproducibly produces the same adverse physiological response regardless of the species.

(iv). Its return reverses the responses.

(v). Physiological responses caused by dietary deficiencies are accompanied by specific biochemical changes.

(vi). Dietary supplementation prevents or reverses these biochemical changes.

1.6.1. Chromium Deficiency.

The manifestations of chromium deficiency include impaired glucose tolerance, elevated fasting, glycosuria, elevated circulating insulin, decreased insulin binding, decreased insulin receptor number, elevated cholesterol and trigylcerides, decreased high density lipoproteins (HDLs), and hypolycemic symptoms, which include drowsiness, shaking, blurred vision, and profuse sweating. Although decreased sperm

count and sterility have been observed in experimental animals, those reproductive pathologies have not been reported in humans.

Wallach (1990), has reported that chromium deficiency in experimental animals impairs growth and fertility, and caused a diabetic like state associated with impaired glucose tolerance, hyperinsulinemia, hypercholesterolemis, and enhanced atherogenesis.

Kieffer (1979), had reported that chromium deficiency can cause eye cataracts in experimental animals. Maternal chromium deficiency has been suggested as a cause of gestational diabetes in humans.

The hypothesis that chromium deficiency can accelerate ageing is supported by data showing that the deficit of bioavailable chromium in processed food and by many studies claiming a progressive decline in body and organ chromium content from birth onwards. Human chromium deficiency has been indisputably proven only in protein-calorie malnutrition and in patients receiving total parental nutrition devoid of chromium supplementation. Polansky et al (1990), reported that stress produced by high-sugar diets, trauma, or exercise will deplete the body of chromium.

1.6.2. Glucose Tolerance Factor.

Glaser and Halpern (1929), who observed that yeast extracts potentiate the action of insulin, were the first to perceive the possible existence of a glucose tolerance factor.

Mertz and Schwartz (1955), postulated the existence of a new dietary agent, the glucose tolerance factor. They observed that when laboratory rats were fed a standard commercial diet, their ability to metabolise sugar became impaired. When chromium containing fractions of yeast or yeast concentrates were added to the rats diets, the blood sugar levels became normal. Trivalent chromium was identified as the active

component of the molecule, the so-called glucose tolerance factor, the exact nature of which was not known. Mertz (1969) postulated that chromium as glucose tolerance factor acts as a co-factor to bind insulin to receptor sites on membranes and therefore enhances the efficiency of insulin. Dietary chromium had been confirmed as a component of glucose tolerance factor, which improves glucose intolerance in adults, especially the elderly. It has been suggested that the glucose tolerance factor, contains two molecules of nicotinic acid and a small oligopeptide such as glutathione, co-ordinated to trivalent chromium. This dinicotinato-amino acid-chromium complex is found in many animal tissues and may be synthesised in the liver.

1.6.3. Chromium Dietary Supplementation.

Improved glucose tolerance in adults and elderly people was demonstrated following daily supplementation with 150-250 μg of chromium as chromic chloride. The effect of chromic chloride supplementation in adult humans took days to weeks to become noticeable, and this interval could not be reduced by increasing the dose. In constrast, Abraham et al (1992), conducted a study in non insulin dependant diabetics and age and sex matched controls. They received either placebo or 250μg of chromium as chromium chloride in $5cm^3$ of syrup. This dose level produced a fourfold to fivefold increase in serum levels.

Animal studies, however, suggested that simple chromium salts, regardless of valence state, did not meet the credentials of an essential element. Many of these salts were not absorbed by the foetus in utero, the requirement of the developing foetus for high concentrations notwithstanding. Intestinal absorption of simple chromium compounds was not dependant on nutritional chromium status of the animal, and the

rate of elimination was not influenced by superimposed injection of high doses of chromium chloride.

It has been postulated that chromium compounds in foods must be absorbed better than simple chromium compounds. Chromium extracted from Brewers yeast was shown to be better absorbed than chromic chloride by the rat. Tuman et al (1978), have reported that most people and animals can convert inorganic chromium to a suitable biologically active form. However, genetically diabetic mice and possibly brittle diabetics and/or people with advanced stages of maturity onset diabetes lose the ability to convert chromium to a usable form and are therefore dependant on preformed, physiologically active forms of chromium. This biologically active chromium (BAC) is trivalent, and it is incorporated into the glucose tolerance factor. (Katz (1994)).

1.7. Physical and Chemical Properties of Chromium.

Elemental chromium was first prepared and characterised by Louis Vanquelin in 1979. In compounds, chromium demonstrates oxidation numbers of 2+, 3+, 4+, 5+, and 6+. The compounds of the trivalent chromium are the most stable and the most abundant. The hexavalent chromium compounds are well known as laboratory reagents and manufacturing intermediates.

1.7.1. Trivalent Chromium Compounds.

The major chemical properties of trivalent chromium compounds in aqueous solution are characterised by the stability of the green hexaaquochromium (III) ion, $[Cr(H_2O)_6]^{3+}$, and the tendency of this ion to precipitate as polymers formed through oxo and/or hydroxo bridging. The electronic configuration of trivalent chromium is

$$1s^2, 2s^2, 2p^6, 3s^2, 3p^6, 3d^3.$$

In aqueous solution, the hexaaquochromium (III) ion, demonstrates the octahedral geometry of d^2sp^3 hybridisation and the kinetic inertness towards ligand exchange of the t_{2g}^3 state. As the pH of the aqueous system is raised, the hexaaquochromium (III) ion, an acid with a pKa of approximately 4, is neutralised to species such as $[Cr(H_2O)_5(OH)]^{2+}$ and $[Cr(H_2O)_4\ (OH)_2]^+$. These species polymerise through oxo and hydroxo bridging. Further deprotonation and polymerisation produce the hydrated chromium (III) oxide. When freshly precipitated the hydrated Cr(III) readily dissolves in both acids and bases;

$$Cr_2O_3.nH_2O + 2OH^- \rightarrow 2CrO_2^- + (n+1)H_2O.$$

$$Cr_2O_3.nH_2O + 6H^+ \rightarrow 2[Cr(H_2O)_6]^{3+} + nH_2O.$$

The hexaaquochromium (III) ion is green, and its absorption spectrum shows maxima at 404 and 570 nm. The molar absorptivities at both wavelengths are low. The absorption maxima change as the co-ordinated water is replaced by other ligands.

Soluble trivalent chromium compounds include $Cr(NO_3)_3.9H_2O$, $CrCl_3.6H_2O$ and $Cr_2(SO_4)_3.18H_20$.

1.7.1.(a). Chromium (III) Complexes with Inorganic Ligands.

Cr(III) accepts electron pairs from many ligands to form a wide variety of co-ordinated compounds. The resulting complexes may be cationic, neutral, or anionic, and essentially all of them are hexaco-ordinated. Once formed, complexes of trivalent chromium are quite stable. Ligand displacement, substitution, or exchange reactions are slow. Half-times of tens of hundreds are not uncommon. Trivalent chromium compounds with ammonia molecules and cyanide ions are also known. $NH_4[Cr(NH_3)_2(CNS)_4]$, Reineke's salt, is a precipitant for amines that contains mixed

ligands. Complexes with polydentate ligands such as ethlenediaminetetraacetate (EDTA) ions or oxalate ions have also been prepared and characterised.

The formation of anionic complexes has been proposed as an explanation for the variations observed in the transport of trivalent chromium into and through the epidermis from aqueous solutions of chromium (III) chloride, chromium (III) sulphate, and chromium (III) nitrate.

1.7.1.(b). Chromium (III) Complexes with Bioligands.

Basic nitrogen, oxygen and sulphur atoms in amino acids, proteins, and other compounds having biological activity have potential electron pair donors for the formation of co-ordination complexes with trivalent chromium. Many such complexes have been prepared and characterised.

Samitz and Katz (1964), have identified trivalent chromium complexes with aspartic acid, glutamic acid, isoleucine, methionine, proline, norvaline, and threonine from spectral observations of chromium (III) nitrate solutions incubated with 10-fold molar excesses of the ligand for 48 hours.

1.7.2. Hexavalent Chromium Compounds.

The ground state electron configuration of the hexavalent atom is

$$1s^2, 2s^2, 2p^6, 3s^2, 3p^6, 4s^1, 3d^5.$$

Divalent chromium compounds are basic, trivalent chromium compounds are amphoteric, and hexavalent chromium compounds are acidic. The acid anhydride (CrO_3), the acid chloride (CrO_2Cl_2), and a wide variety of metal chromates ($MCrO_4$) and metal dichromates (MCr_2O_7) are typical hexavalent chromium compounds. The acid functions have been evaluated.

$$H_2CrO_4 \rightarrow H^+ + HCrO_4^- \qquad K_{a1} = [H^+][HCrO_4^-] / [H_2CrO_4]$$

$$HCrO_4 \rightarrow H^+ + CrO_4^{2-} \qquad K_{a2} = [H^+][CrO_4^{2-}] / [HCrO_4]$$

as has the chromate-dichromate equilibrium.

$$2HCrO_4 \Leftrightarrow Cr_2O_7^{2-} + H_2O \qquad K_{eq1} = [Cr_2O_7^{2-}] / [HCrO_4^-]$$

The chromate ion is tetrahedral, and the structure of the dichromate ion corresponds to two tetrahadra linked through a corner oxygen. The Cr-O-Cr bond angles in the polymeric species are about 126°C, and the Cr-O bond lengths of the linking Cr-O-Cr bonds are 179pm, while the Cr-O bond lengths in the chromate ion and in the dichromate ion are 166 and 163 pm, respectively. (figure 1.5).

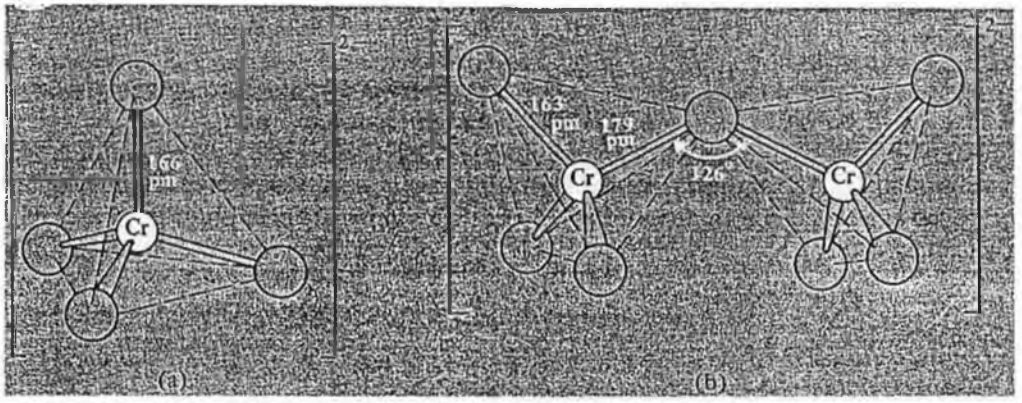


Figure1.5. (a). CrO_4^{2-} ion and (b) $Cr_2O_7^{2-}$ ion.

Aqueous solutions of hexavalent chromium compounds absorb in the ultraviolet and violet regions of the spectrum. Those of the chromates are distinctly yellow, dichromates are orange, and the higher polymers are red. Aqueous solutions of potassium chromate absorb strongly at wavelengths of 370-375nm and demonstrate a molar absorptivity of 4.5 x 10^{-3}L / (mol^{-1} cm^{-1}). Aqueous solutions of potassium dichromate show absorption maxima near 350 and near 450nm. The molar absorptivities are 2.5 x 10^3 and 3.7 x 10^2L / ($mol^{-1}cm^{-1}$), respectively.

The solubility equilibria of hexavalent chromium compounds are complex and pH dependant. Heavy metal dichromates are usually more soluble than the corresponding metal chromates.

1.7.3. Chromium Compounds of Other Oxidation State.

Both the pentavalent and the tetravalent oxidation states of chromium are formed as transient intermediates in the reduction of hexavalent chromium to trivalent chromium. Although both these oxidation states of chromium are thermodynamically unstable, some of their insoluble and co-ordinated compounds are long-lived.

1.7.3.(a). Pentavalent Chromium Compounds.

The electronic configuration of pentavalent chromium is

$$1s^2, 2s^2, 2p^6, 3s^3, 3p^6, 3d^1.$$

These compounds are paramagnetic because of the unpaired, $3d^1$, electron. Consequently, many of the transient intermediate pentavalent chromium compounds can be observed by electron spin resonance/electron paramagnetic resonance (ESR/EPR) spectroscopy.

Potassium perchromate, or potassium tetraperoxochromate (VI), contains the octaco-ordinate, dodecahedral $[Cr(O_2)_4]^{3-}$ ion. The O_2 groups are unsymmetrically co-ordinated, with Cr-O bond lengths of 185 and 195pm. The O-O bond length is 141pm.

1.7.3.(b). Tetravalent Chromium Compounds.

The best known of the tetravalent chromium compounds, CrO_2, is ferromagnetic. It is used to make high quality recording tape.

1.7.3.(c). Divalent Chromium Compounds.

Compounds of divalent chromium are vulnerable to air oxidation. The anhydrous divalent salts can be preserved in nitrogen, carbon dioxide, or other inert atmosphere. In aqueous solution, the divalent chromium ion is a powerful reducing agent. In acidic solution it reduces water to liberate hydrogen:

$$2[Cr(H_2O)_6]^{2+} \rightarrow 2[Cr(OH)(H_2O)_5]^{2+} + H_2$$

Chromium (II) acetate dihydrate is among the most stable divalent chromium compound. $Cr_2(CH_3COO)_4.2H_2O$ has an unusual dimeric bridge structure in which each Cr(II) is surrounded by a distorted octahedron made up of four oxygens from the four bidentate acetate ions, one oxygen from one of the water molecules, and the other chromium (II). The four acetate ions bridge between the two of the chromium (II) and there is evidence of chromium to chromium bonding. The chromium to chromium distance is only 2.36pm, and chromium (II) acetate dihydrate is diamagnetic. The electronic configuration of chromium (II) is

$$1s^2, 2s^2, 2p^6, 3s^2, 3p^6, 3d^4.$$

which indicates four unpaired electrons. (Katz (1994)).

1.7.4. Oxides of Chromium.

CrO_3 is a strongly acidic and rather basic covalent oxide of chromium with a melting point of only 197°C. Its deep red crystals are made up of chains of corner-shared CrO_4, tetrahedra. It is commonly called 'chromic acid' and is generally prepared by the addition of concentrated H_2SO_4 to a saturated aqueous solution of a dichromate. Its strong oxidising properties are widely used in organic chemistry. CrO_3 melts with some decomposition and, if heated above 220-250°C, it loses oxygen to give a succession of lower oxides until the green Cr_2O_3 is formed.

Cr_2O_3 has the corundum structure and it finds wide application as a green pigment. It is a semi-conductor and is anti-ferromagnetic below 35°C. Cr_2O_3 is the most stable oxide of chromium and is the final product of combustion of the metal, though it is more conveniently obtained by heating ammonium dichromate.

$$(NH_4)_2Cr_2O_7 \rightarrow Cr_2O_3 + N_2 + 4H_2O$$

When produced by this method it is unreactive, but, if precipitated as the hydrous oxide (or hydroxide) from aqueous Cr(III) solutions it is amphoteric. It readily dissolves in acid to give an extensive cationic chemistry based on the $[Cr(H_2O)_6]^{3+}$ ion, and in alkalis it produces complicated, extensively hydrated chromate (III) species ("chromites").

The third major oxide of chromium is the brown-black, CrO_2, which is an intermediate product in the decomposition of CrO_3 to Cr_2O_3, and has a rutile structure. It has metallic conductivity and its ferromagnetic properties lead to its commercial importance in the manufacture of magnetic recording tape.

1.7.5. Halides and Oxohalides of Chromium.

The group oxidation state of +6 is attained by chromium only with the strongly oxidising fluorine. This is also true for the +5 oxidation state and in the +4 oxidation state the iodides have a doubtful or unstable existence. In lower oxidation states all the chromium halides are known. In the oxohalides (which are largely confined to the +6 and +5 oxidation states) chromium forms an oxofluoride in the lower of these oxidation states.

For the preparation of the yellow CrF_6, temperatures of 400°C and a pressure of 200-300 atms are required for its formation and reduction of the pressure causes it to dissociate into CrF_5 and F_2 even at temperatures as low as –100°C.

Of the pentahalides, chromium forms only the fluoride, which is a strongly oxidising, bright red, volatile solid prepared from the elements using less severe conditions than for CrF_6.

The most stable representative of the tetrahalides are the fluorides, (CrF_4, $CrCl_4$, $CrBr_4$ and CrI_4), CrF_4 is an unreactive solid which melts without decomposition at 277°C. All four of the chromium trihalides are known (CrF_3, $CrCl_3$, $CrBr_3$ and CrI_3), this being much the most stable oxidation state for chromium. They can be prepared by reacting the halogen and the metal, though CrF_3 is better obtained from HF and $CrCl_3$ at 500°C. The fluoride is green, the chloride red-violet, and the bromide and the iodide are dark green to black. In all cases a layer structure leads to octahedral co-ordination of the metal.

Anhydrous chromium dihalides (CrF_2, $CrCl_2$, $CrBr_2$ and CrI_2), are conveniently prepared by reduction of the trihalide with H_2 at 300-500°C. They are all deliquescent and the hydrates can be obtained by reduction of the trihalides using pure chromium metal and aqueous HX. All have distorted octahedral structures.

The oxohalides of chromium are very susceptible to hydrolysis. They are yellow to red liquids or volatile solids, the best known is the deep-red liquid, chromyl chloride, CrO_2Cl_2. It is most commonly encountered as the distillate in quantitative tests for chromium or chloride and can be obtained by heating a dichromate and chloride in concentrated H_2SO_4, it is an extremely aggressive oxidising agent.

1.7.6. Sulphides, Selenides, and Tellurides of Chromium.

The sulphides, though showing some similarities in stoichiometry to the principle oxides, tend to be more stable in the lower oxidation states of the metal. Thus, chromium forms no trisulphides. Cr_2S_3 is formed by heating powdered chromium

with sulphur, or by the action of $H_2S(g)$ on Cr_2O_3, $CrCl_3$ or chromium. It decomposes to CrS on being heated, by a number of intermediate phases, which approximate in decomposition to Cr_3S_4, Cr_5S_6 and Cr_7S_8. The structural relationship between these various phases can be elegantly related to the CdI_2-NiAs structure motif (figure 1.6).

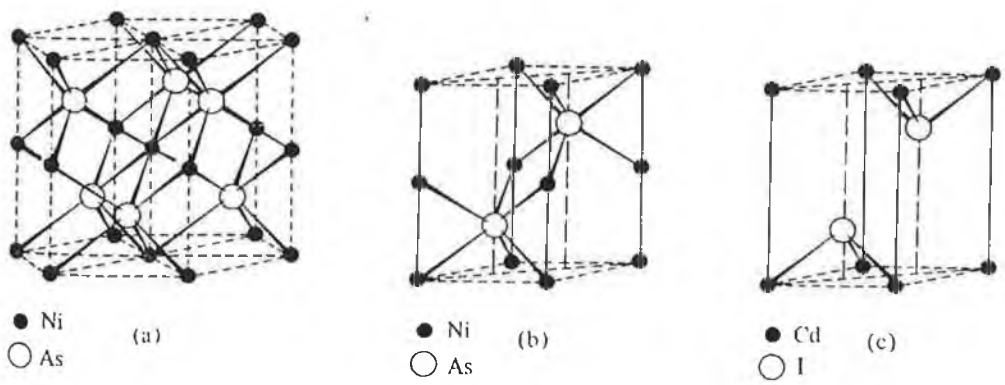


Figure 1.6. CdI_2-NiAs structure motif.

Removal of M atoms from alternative layers of the NiAs structure yields the CdI_2 layer lattice. Thus, in Cr_7S_8 one quarter of the chromium atoms in every second metal layer are randomly removed. With Cr_5S_6 one third of the chromium atoms in every second metal layer are absent in an ordered way and with Cr_2S_3 two thirds of the chromium atoms in every second metal layer also absent in an ordered way. Because of the stringent geometry criteria for the ordering, these two phases are truly stoichiometric and show no detectable range of composition variation. By contrast, in Cr_3S_4 every second chromium atom in alternate layers is missing but, because it is not possible for every second atom in a trigonal array to be missing in a regular pattern and still possess trigonal symmetry, there is some disordering, the symmetry is lowered, and a small range of composition variation is permitted. Of these various phases Cr_2S_3 and CrS are semi-conductors, whereas Cr_7S_8, Cr_5S_6 and Cr_3S_4 are metallic, and all exhibit magnetic ordering. The corresponding selenides CrSe,

Cr_7Se_8, Cr_3Se_4, Cr_2Se_3, Cr_5Se_8 and Cr_7Se_{12} are broadly similar, as are the tellurides CrTe, Cr_7Te_8, Cr_5Te_6, Cr_3Te_4, Cr_2Te_3, and Cr_5Te_8. (Greenwood (1984)).

1.8. Buffer Solutions.

A solution consisting of a weak acid along with a conjugate base is called a buffer solution because it resists pH changes when diluted (or perhaps concentrated) or when various amounts of acid or base are added. But, there is also a definite limit called the buffer capacity which defines how much acid or base can be added to a given buffer solution before any appreciable change in pH results. This buffer capacity is set by the original amounts of the weak acid and its conjugate, since one or the other is consumed by reaction with the added acid or base.

1.8.1. Factors Affecting the Choice of a Buffer.

The buffering ability of a weakly acidic or basic group is limited approximately to the range $pH = pK_a$ +/- 1, the greatest effect being $pH = pK_a$. This is the most important single factor in choosing a buffer for any particular application. However, there are usually a number of other considerations, such as the nature of the reaction system, the ionic strength, the effects of temperature and dilution, the possibility of forming insoluble or strongly coloured species or otherwise interacting with components of the solution. For example, the effect of temperature on pK_a of the buffer should be known and, preferably should not be very large. This is an important factor for buffers based on Tris (tris(hydroxymethyl)aminomethane) and aliphatic amines. The buffer species should also be chemically stable, should be readily soluble in water and not be readily extracted by organic solvents.

A good buffer shows little change in pH if accidentally contaminated with acidic or basic materials. For a buffer of the type $BH^+ \Leftrightarrow B + H^+$, acidic contaminants have little effects if they are weaker acids than BH^+. Similarly, basic contaminants that are weaker bases than B are not important. For measurements of rates or of physical constants it is often desirable to work under conditions of known low ionic strength. In all cases, the pH of a buffered medium should be determined only after all components have been added and at the temperature of the final measurements.

1.8.2. Practical Limitations in the Use of Buffers.

The suitability of a buffer system for any particular application depends on many factors. Not only must the buffer species be appreciably soluble in water, but it is also important that they do not react with ions or molecules present in the solution. For example, phosphate and pyrophosphate buffers are unsuitable if a solution contains calcium or certain other di- or trivalent cations which form insoluble phosphates, or if reaction progress is followed by a change in phosphate content. Similarly, carbonate buffers can precipitate calcium ions.

A difficulty in using bicarbonate/CO_2 buffers is that pH consistency requires the use of closed systems equilibrated with a controlled level of CO_2 in the gas phase. Otherwise, loss of CO_2 from the solution can lead to a progressive and undesirable rise in pH.

On prolonged storage, borax is likely to lose some of its water of crystallisation unless the container is tightly stoppered. This is not serious when borax is used, on its own, as a buffer standard but it can lead to significant errors in buffers when its strength as an acid or a base is important. The storage of alkalis and alkaline buffer solutions presents problems of the rapidity with which CO_2 is absorbed and because of the slow

attack by reagents on the bottles in which they are stored. With phosphate solutions this can lead to the deposition of calcium phosphate.

Where the buffer has metal-complexing ability, problems may arise, particularly if heavy metal ions are present. Competition between metal ions and protons for attachment to buffer species can lead to a lowering of pH. Other factors which need to be allowed for are the change in pH with dilution of a buffer and, more importantly, the effect of temperature on pK_a of a buffer. Tris has a pK_a of 8.85 at 0°C, 8.06 at 25°C and 7.72 at 37°C, so that the pH of the Tris buffer can fall by more than 1 pH unit in warming from 0°C to 37°C. Comparable effects are found for other cationic buffers as the aliphatic amines.

1.8.3. Biological Effects.

Buffer systems can exert effects on biological systems in three main ways. They may specifically stimulate or depress enzyme activity. They may interfere or react with substrates, inhibitors or cofactors. Non-specifically they may exert effects because of their ionic strength. In general, buffer concentrations should be kept as low as possible, having regard for the need to maintain pH constancy, and the medium should be adjusted by adding appropriate inorganic and organic ions to simulate physiological conditions.

Some common examples of enzymes inhibited by phosphate ions include carboxypeptidase, fumarase, urease, phosphoglucomutase, carboxylase, arlysulphatase and muscle deaminase. Frequently this inhibition is due to competition of the phosphate with substrates containing phosphate groups or to complex formation with a metal ion essential for the enzyme activity.

Tris(hydroxymethyl)aminomethane has been a major biochemical buffer for many years, partly because it is relatively inexpensive and readily available in a highly purified form. However, it has disadvantages. These include its reactivity as a primary amine and its appreciable solubility in inorganic solvents which leads to its accumulation in the biological phases of reaction systems. Thus, Tris buffer displaces the electron transport-, and phosphorylation-, pH rate profiles for chloroplasts by almost a pH unit when compared with a number of other buffers. It also inhibits isocitrate dehydrogenase of pea mitochondria whereas HEPES does not. (Perrin (1974)).

Sirinawin et al (1997), used tricaprylmethyl ammonium chloride (Aliquot-336) for the extraction of Cr(VI) into methyl isobutyl ketone (MIBK). The basic principle of the extraction was ion-pair extraction of Cr(VI) from an acidic solution. They found that the extraction was affected by the concentration of chloride, nitrate and sulphate, as well as pH. The most favourable pH-range was between 1 and 3. An extraction time of 2 minutes was found to be optimal. Shorter extraction times gave lower recovery, as did longer extraction times.

Flores-Veles et al (1994), used Tris (tris(hydroxymethyl)aminomethane) for the extraction of Cr(VI) from soil samples at pH 9.85 and pH 13. They found that at pH 9.85 the Tris solution did not extract 100% of the Cr(VI) added to the soil. The percentage recovery obtained was lower than 90%. They found that by increasing the pH of the Tris solution to 13 a percentage recovery of 98.6% was obtained. This meant that strong alkaline media are efficient for the desorption of chromium in soils.

1.9. METHODS OF DETERMINATION OF Cr(VI).

The four main methods of detection of Cr(VI) used in this thesis are

(i). Voltammetry.

(ii). G.F.A.A. Spectroscopy.

(iii). U.V. Spectroscopy.

(iv). H.P.L.C.

Outlined here are the main principles on which these instruments work and an overview of the reactions involved and previous works carried out using these methods.

1.9.(a). Voltammetry.

Principle.

In an electrolysis cell the current produced in the cell is a measure of the amount of chemical change occurring at the electrodes. This is a well known principle embodied in Faraday's Laws. In order for a reaction to occur at an electrode the electrode potential must exceed a critical value. There are a variety of electro-analytical techniques which are designed to measure cell current as a function of electrode potential. These techniques also belong to the branch of the electro-analytical techniques family known as voltammetry at finite current. If the electrode potential is changed in a linear mode then we have linear sweep voltammetry (LSV). Linear sweep voltammetry may be carried out using a variety of electrode materials such as gold, platinum, carbon and also mercury. If, and only if, mercury in the form of a dropping mercury electrode (DME) is used as the working electrode, we have polarography. The working electrode (WE) in a cell is the electrode where the reaction of interest in occurring. The mercury electrode in polarography takes a

special form consisting of small droplets of mercury generated at the lower end of a glass tube. This is what is termed the dropping mercury electrode (DME).

Polarography is then an example of voltammetry at controlled potential in which the working electrode consists of a dropping mercury electrode and the potential of the working electrode is changed in a linear mode. The Czechoslovakian chemist Jaroslav Hegrovsky discovered, reported and established this technique in 1922.

The Dropping Mercury Electrode.

Mercury is very toxic and demands a neat and tidy working practice. It is a liquid under normal experimental conditions and thus, presents a smooth homogeneous surface to the solution. It is a good electrical conductor and provides a surface for the required electrode reaction. A good electrode material provides a high voltage limit in anodic and cathodic directions and thus, a wide voltage window for analysis. The voltage window for DME is usually from about +0.2V (SCE) to -2.0V (SCE) with small variations due to differing solution conditions.

Advantages of the DME are;

(a). The drops are reproducible in size and each drop grows in an environment identical to that of its predecessor giving rise to reproducible currents for the same conditions.

(b). A new surface is presented with each drop so that no appreciable accumulation of reaction product at the surface can occur either as adsorbed species or as precipitates.

(c). Species present in the solution, other than those involved in the electrode reaction, are also unable to adsorb to any extent during the lifetime of the drop.

(d). Electrolysis is so small that solutions may be analysed many times with no appreciable change in the analyte concentration.

(e). The over potential for the reduction of the hydronium ion is high so that interference's from reduction of this species do not occur until potentials more negative than -1.6V are reached.

Limitations of this electrode would include;

(a). The voltage limit of 0.2V to -2.0V. The use of non-aqueous solvents and supporting electrolytes such as tetraalkylammonium salts enable the negative limit to be extended to -3V but little can be done about the anodic limit which is determined by the tendency of the mercury to oxidise at potentials in the range 0.0V to 0.2V.

(b). The high detection limit. Polarography is best used for metallic cations in the range 10^{-2} to 10^{-4} mol dm^{-3}. At concentrations greater than 10^{-2} mol dm^{-3} the large change in concentration at the electrode surface causes the current to become erratic. At concentrations below 10^{-4} mol dm^{-3} the capacitative current becomes comparable with the diffusion controlled Faradaic current.

(c). Catalytic hydrogen currents. These are caused by organic species adsorbed on the electrode that are capable of protonation.

(d). Adsorption currents. These occur if the product of the reaction adsorbs strongly or if the product is insoluble, it simply adheres to the surface.

(e). Kinetic currents. These are produced if the reactant or product is also to participate in a non-electrochemical reaction in the solution.

All of these effects can cause either distortion of the expected analyte wave or extra waves to appear, sometimes preceding and sometimes following the analyte wave.

The DME is operated at negatively applied potentials, so that reduction occurs and (negative) cathodic waves are produced. If positive waves are applied, oxidation occurs producing anodic (positive) waves. The DME consists of a resevoir of mercury that is pushed through a thin capillary under pressure. A hammer at the top of the capillary controls the drop rate of the mercury. When the drops emerge in the solution they become the working electrode.

During the recording of a polarogram the applied voltage is gradually increased, so that the potential of the working electrode becomes more negative. When the applied potential becomes large enough that the applied electro-motive force (emf) is greater than the back emf of the cell, an electrochemical reaction occurs at the working electrode and currents begin to flow through the cell.

1. Pulse Polarographic Techniques.

Two techniques, normal and differential pulse polarography, are discussed here. These use a complex applied voltage signal and current sampling techniques to minimise interfering background signals and to maximise the analytical signal, giving a greater increased sensitivity. The major drawback of dc (direct current) polarography is that of the signal to noise ratio, which is unwanted instrumental signal unrelated to the analyte or its concentration, often random and uncertain. With dc polarography the signal is the mean Faradaic current and the principle source of noise is the capative current for the growing mercury drop. The signal to noise ratio, that is the ratio of the Faradaic current to the capacitative current is greatest at the end of the drop lifetime. Classical dc polarography records the mean current during the drop lifetime and thus does not record the maximum Faradaic current. It also includes an

appreciable contribution from the capacitative current, which leads to a limit of detection of about 5 x 10^{-5} mol dm^{-3}.

A. Normal Pulse Polarography. (NPP).

The basis of this type of polarography is that the potential is kept at a lower voltage until the moment of measurement, thus preventing electrolysis during most of the drop lifetime. The potential is kept at a suitable constant base throughout the drop lifetime and the chosen potential signal is imposed as a very short pulse (about 60mS) near the end of the drop lifetime, causing very little electrolysis and depletion. The overall form of the applied voltage signal is a series of potential pulses, one to each drop rising in a linear ramp with a base potential maintained between the pulses. The current recorded at the end of each pulse is plotted against the potential of the pulse. The resultant current/potential curve is similar in form to the classical dc polarographic curve but the noise, i.e. the capacitative current, has been dramatically reduced. Thus the sensitivity of the technique has been improved by about two orders of magnitude giving a limit of detection of about 10^{-7} mol dm^{-3}.

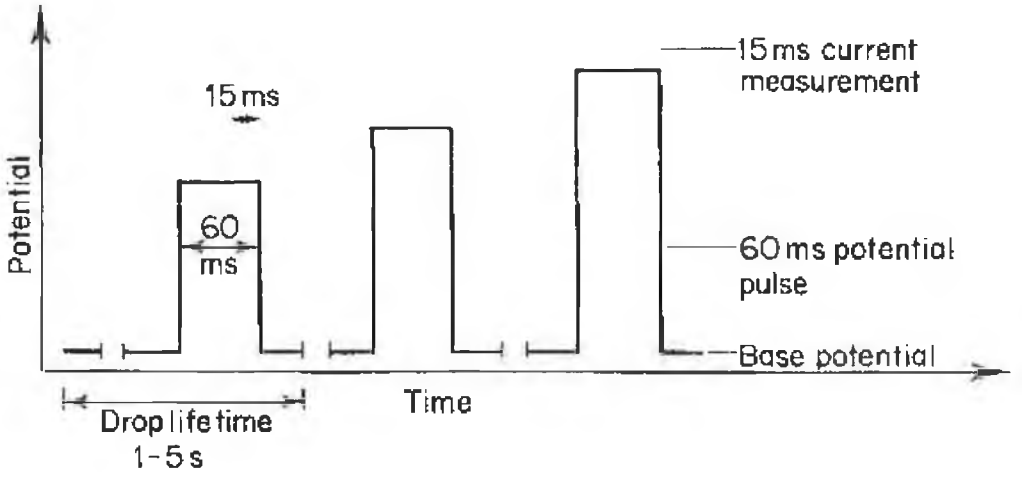


Figure 1.7. Profile of the Potential Pulse and Current Measurement in NPP.

B. Differential Pulse Polarography. (DPP).

This technique differs from NPP in that after the potential pulse the potential does not return to a constant base value. Instead the potential pulse is of a small constant amplitude (10-100mV) and is superimposed on a conventional rising linear dc voltage ramp. Again the pulse is imposed for about 60mS near the end of the drop lifetime when the growth of the drop has almost ceased. The current is measured in two intervals of about 15mS, the first immediately prior to the potential pulse and the second during but near the end of the potential pulse. The final current signal displayed is then, in fact, the difference of these two current values. This technique produces not a wave but a peak with the maximum current signal at roughly the half wave potential of the classical dc and NP polarography. Electrolysis and depletion occur throughout the drop lifetime with this technique, leading to DPP being typically an order of magnitude more sensitive than NPP, giving a limit of detection of 10^{-7} - 10^{-8} mol dm^{-3}.

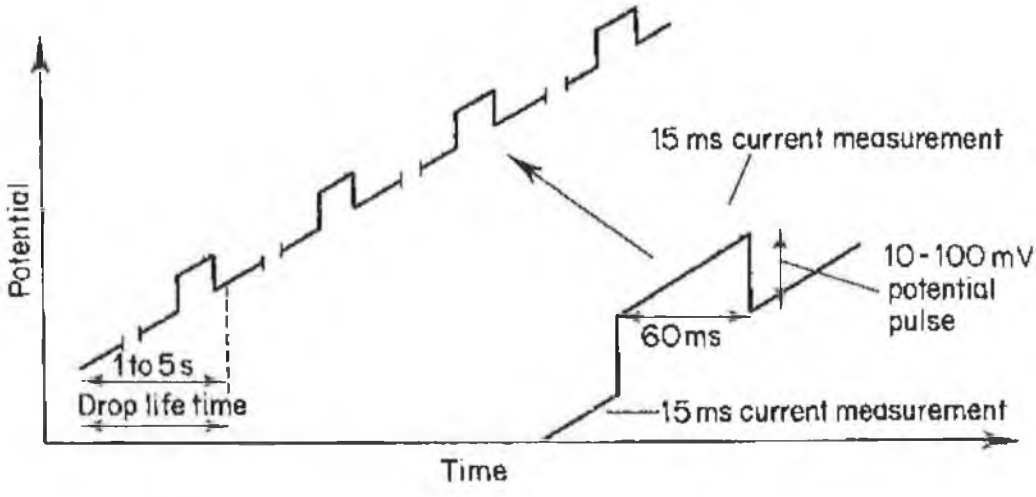


Figure 1.8. Profile of the Potential Pulse and Current Measurement in DPP.

2. Stripping Voltammetry.

Although DPP is the most sensitive direct polarographic technique, an even greater sensitivity can be obtained by employing stripping voltammetry. This method is applicable to a limited number of important analytes and requires the use of solid or stationary electrodes. The preconcentration or deposition step consists of the controlled electro-deposition, at a fixed potential, of the species of interest onto the stationary electrode. Since the volume of mercury is small compared to the volume of solution deposited on the electrode preconcentration occurs. This is followed by the determination step, which consists of electrolytically stripping the deposited species back into solution.

The main steps involved in stripping voltammetry are;

1. The Deposition Step.

The most popular electrodes are the hanging mercury drop electrode (HMDE), and the mercury film electrode (MFE) supported on gold or platinum. In metal ion analysis, the mercury electrodes offer the advantage that the deposition product, the metal, dissolves in the mercury to form a liquid amalgam. This offers better reproducibility. The MFE is capable of more sensitive measurements than the HMDE but is less suited to relatively higher trace amounts, since the solubility capacity of a film is less.

(a). Stirring.

The stirring of the solution during the deposition step increases the rate at which the analyte reaches the electrode to be deposited. The stirring must be uniform and at a rigidly controlled rate, and it must be gentle otherwise unpredictable eddy effects will occur. Deposition in a still, unstirred solution might appear to offer a much higher reproducibility of conditions but at the cost of a much reduced sensitivity and a much longer deposition time. An alternative to stirring a solution is to rotate the electrode,

where the electrode usually consists of a rod cut to expose a flat disc. The rod is then rotated about its axis and is usually made of glassy graphite, platinum or gold.

(b). Deposition Time.

The greatest sensitivity would clearly be obtained by carrying on the deposition process until all the analyte would be deposited on the electrode. But it is best to avoid long deposition times as they often lead to various complications resulting in a loss of proportionality between final signal and the concentration of the analyte. In general a good guide is to choose a deposition time so that only about 2% of the total analyte is depleted from solution, this allows the rest of the solution to remain unaltered.

(c). Deposition Potential.

Usually a potential is chosen a few hundred millivolts larger than the polarographic half wave potential of the analyte. In the analysis of a solution containing a number of metal ions, each metal in it will have its own individual deposition potential. Thus, only one metal or a group of metals can be deposited, avoiding the deposition of other metals which might interfere with the stripping step. The higher the potential, the more types of metals will be deposited and the more interference's likely.

2. The Stripping Step.

There are two stripping procedures of great significance;

(a). Direct Current (DC) or Linear Sweep Stripping Voltammetry.

This being the simplest of the procedures involves the imposition of a simple linear voltage scan on the electrode.

(i). DC Anodic Stripping Voltammetry. For metal ions a cathodic deposition potential (negative) is set for the depositon time reducing the ions to metal. Then the linear

voltage scan is started with the potential moving towards anodic potentials (positive) for the re-oxidation of the metal.

(ii). DC Cathodic Stripping Voltammetry. For anodic species an anodic deposition potential (positive) is set oxidising the ions to metal and the stripping step is a voltage scan to cathodic potentials (negative).

As the potential scan begins no current initially flows. But when the re-oxidation potential (or re-reduction potential) is reached the current rapidly rises. There is only a fixed amount of material deposited in the deposition step. The current must therefore fall back towards the base line as the last of the deposited material is re-oxidised (or re-reduced). The dc stripping signal thus consists of a peak, the height of which is used to determine the concentration in the solution.

(b). Differential Pulse Stripping Voltammetry.

The voltage sweep used here is identical to that used in conventional DPP. The current is sampled just before the pulse and almost at the end of the pulse. The first signal is the difference of these 2 current values. At the steeply rising stripping peak this small change in the potential will produce a large change in the electrolysis current whereas, the small change in potential will produce only a very small effect on capative current and other sources of noise. This produces a much greater sensitivity and allows the use of much shorter pre-electrolysis or deposition time. It is because of the greater sensitivity of this technique that it can sometimes even allow the use of a pre-electrolysis deposition step in an unstirred solution, thus avoiding the problems of the reproducibility of the stirring. For concentrations below 50ppb stirring is essential but at higher concentrations it can sometimes be avoided.

A. Anodic Stripping Voltammetry.

This is concerned almost entirely with trace metal analysis. Metal ions lend themselves particularly well to stripping voltammetry. The metal deposited in the deposition step generally dissolves in the mercury drop to form an amalgam. This avoids any problems with the nature of an insoluble deposit. If more than one metal ion is deposited they will appear as separate peaks at different potentials in the stripping step. However the formation of intermetallic compounds can cause problems. When zinc and copper are present in solution there is a tendency to form a Zn/Cu intermetallic compound, when large amounts are deposited at the mercury electrode. When an intermetallic compound is formed the stripping peaks for the constituent metals may be shifted, severely depressed, or even be absent altogether. When an alloy is formed at a solid electrode its dissolution potentials, in the stripping step, may be quite different to those of the constituent metals. Such interfering effects can be minimised or avoided by reducing the deposition time and the total amount of metal deposited. This would lead to a loss in sensitivity but the case of differential stripping voltammetry can effect this.

B. Cathodic Stripping Voltammetry.

The most common species determined by cathodic stripping voltammetry are ions such as halides or sulphides, at a mercury electrode. This technique involves the formation of a film of mercury (I) salts on the electrode in the deposition step. The anodic oxidation process involved in the deposition step is in fact the oxidation of mercury (I) ions, which immediately precipitate insoluble mercury (I) salts with the halide ion, onto the surface of the electrode. The subsequent cathodic stripping peak for the mercury (I) salt of each anion has its own individual potential. Cathodic

stripping voltammetry has proved suitable for a number of organic compounds, including drugs and pesticides, which generally contain sulphur and again the deposition step involves anodic (oxidation) formation of an insoluble mercury salt. This method is a highly sensitive one but is suited only to very low levels. (Riley (1987)).

Wang and Lu (1992) used catalytic stripping voltammetry in the presence of cupferron for the measurement of ultra trace levels of chromium. Optimum conditions were obtained at 1 x 10^{-8} mol dm^{-3} piperazine-N,N'-bis(ethansulphonic acid) (PIPES) solution at pH 7 containing 1 x10^{-4} mol dm^{-3} cupferron. A potential of -0.82V was used because it provided the best signal-to-background characteristics. Cupferron served as a complexing agent and also as an oxidising agent. The strong adsorption of the chromium-cupferron complex was used as an effective preconcentration step, prior to voltammetric measurements. It was discovered that the longer the preconcentration time, the more the metal complex is adsorbed and the larger the peak current. Using this procedure measurements of parts per trillion was feasible and a detection limit of 1.0ng dm^{-3} was obtained.

Vukomanovic and vanLoon (1997) employed adsorptive stripping voltammetry with pyrocatechol violet (PCV) for the measurement of Cr(III) and Cr(VI) in aqueous solutions. They found that a blank containing 6 mmol dm^{-3} acetate buffer and 0.5µmol dm^{-3} PCV gave a peak at a potential of -0.53V, and on addition of Cr(VI) this reduction peak decreased while a new reduction peak at -0.73V increased as the concentration of Cr(VI) added increased. It was found that the growth of the Cr(VI)-PCV reduction peak ceased when the ratio of Cr(VI) to PCV was approximately

equimolar. They also found that no Cr(III) -PCV peak was observed. Maximum separation between reduction peaks of uncomplexed PCV and Cr-PCV was observed at pH 6.7. Total chromium was determined by oxidation of Cr(III) to Cr(VI) by UV irradiation and an oxygen saturated solution. Using this method and a preconcentration time of 1 minute a detection limit of 0.15ppb Cr(VI) was established.

Korolczuk and Grabarczyk (1999) used cyclic voltammetry for the determination of traces of Cr(III) in the presence of bipyridine. Their method is based on the fact that Cr(VI) ions are reduced to Cr(III) after an accumulation of 60s at a potential of -1.0V, and that in the presence of bipyridine the Cr(III)-bipyridine complex is formed. Then during a negative direction scan the complex was reduced to a Cr(II)-bipyridine complex and produced a peak at -1.44V. When the direction of the scan was changed the Cr(II)-bipyridine complex was oxidised to Cr(III)-bipyridine and an anodic peak was produced. A pH of 4.5 was chosen for the supporting electrolyte (acetate / acetic acid buffer) as a higher pH caused the co-precipitation of hydroxide. An optimum accumulation potential of -0.25V and an optimum equilibrium time of 15s were chosen. Using these parameters a linear calibration curve from 0.156ppb to 1.56ppb Cr(VI) was produced giving a detection limit of 0.051ppb for Cr(VI) for an accumulation time of 120s, and for an accumulation time of 600s a linear curve from 0.025ppb to 0.260ppb Cr(VI) was produced with a detection limit of 0.010ppb Cr(VI).

Korolczuk and Grabarczyk (1998) also determined Cr(VI) in reference materials and river water samples spiked with known amounts of Cr(VI) using catalytic currents in the presence of nitrate. Here an optimum deposition potential of -1.65V was chosen

along with an oxidation time of 45s. Any increase in potential or time above those chosen did not cause an increase in the current of the catalytic peak. They also discovered that the minimal deposition time needed for the appearance of the catalytic peak on the voltammogram (t_o) is inversely proportional to the Cr(VI) concentration. An optimum pH value of 9.3 was chosen and using these conditions a linear calibration curve was obtained from 0.026ppb to 0.260ppb Cr(VI). The reference material was analysed using these parameters and the results obtained agreed with the reference value. From this result and from the results obtained from the river water samples analysed this method can be used for Cr(VI) and total chromium determinations.

Cr(III) ions form, in aqueous solutions, strong complexes with ligands such as EDTA, CDTA and DTPA, which are then reduced at the dropping mercury electrode (DME) giving well defined polarograms. Zarebski (1977) used NP and DP polarography to obtain polarograms. He discovered that Cr(III) complexes were reduced at the DME at relatively high potentials (about -1.2V) and that the wave of hydrogen was remarkably close to the wave of chromium especially in buffered supporting electrolytes. This lead to the limiting current in DPP to be less defined and for NPP the residual current to be higher especially at concentrations close to the detection limit. Therefore an unbuffered supporting electrolyte was used for the analysis. Using a KCl supporting electrolyte the pH of the solution was found to have a significant influence on the shape and height of the polarograms of Cr(III)-complexes. At pH lower than 5.5 the hydrogen wave interfered with the chromium wave and at pH higher than 6.4 the limiting current decreased leading to an optimum pH of 6.0. Using these conditions a linear curve from 10^{-9} mol dm^{-3} to 10^{-4} mol dm^{-3} was

produced. A higher sensitivity was achieved by use of EDTA or DTPA in a nitrate supporting electrolyte using DPP. The use of such an electrolyte lead to a great increase in its NP and DP polarograms which gave a detection limit of 5.2ppb for both Cr(III) and Cr(VI).

Ghandour and El-Shatoury (1996) also used a nitrate supporting electrolyte for the determination of Cr(VI). By using cathodic linear sweep stripping voltammetry (CLSSV) as low as 0.25ppb Cr(VI) was detected and by using differential pulse cathodic stripping voltammetry (DPCSV) as low as 0.52ppb Cr(VI) was detected. They observed that no adsorptive peak was produced at pH <5 or more than 10 and that the peak height reached its maximum between pH 6.96 and 7.37. An optimum initial potential of +0.10V was chosen as it was observed that the peak height increased as initial potential increased up to 0.0V and reached a maximum value between +0.14V and +0.10V. It was also discovered that the peak height increased with increasing adsorption time up to120s and became constant at 150s. Using these parameters the precision and accuracy were investigated and found to be satisfactory with RSD = 1.24 and coefficient of variation r = 0.998 for 10 replicate analysis.

1.9.(b). Graphite Furnace Atomic Absorption Spectroscopy.

Principle.

The generation of atoms by means of an electrically heated graphite furnace atomiser is a technique which is complementary to conventional flame atomic absorption (A.A.), rather than a technique which replaces it. There are many analytical advantages to flame A.A., but there are many trace metal analyses which are only possible by means of a graphite furnace atomiser. With these two techniques for the generation of ground state atoms, plus the vapour generation method for some selective elements, A.A. remains one of the most versatile yet specific methods for the measurement of metals.

Disadvantages of Flame Atomisation.

(a). The efficiency of pneumatic nebuliser/spray chamber systems is low; typically about 10%.

(b). For the amount of sample finally reaching the flame, the production of atoms in the ground state is governed by many variables such as the flame temperature, interactions between flame gases, matrix components and analyte, chemical interference's, and the extent to which the analyte molecular species are dissociated.

(c). The zone of the flame in which absorption actually occurs is only a small section of the whole flame.

(d). The overall effect of (a), (b) and (c) above is that the number of atoms contributing to the analytical signal is extremely small compared with the total amount of element aspirated.

(e). Flame gases also produce distinct absorption and emission bands which can cause interference's and background noise.

Advantages of Furnace Atomisation.

(a). Sensitivity is better. Graphite furnace atomiser methods are typically 100 times more sensitive than flame methods for most elements in a wide range of samples.

(b). Less sample is needed. Determinations typically can be carried out with only 5μL of sample; up to 100μL may be accepted. Flame methods normally require about $5cm^3$.

(c). Sample preparation can be simplified. Many samples such as concentrated acids, viscous liquids, organic solvents and liquids with high dissolved solids can be analysed directly on the graphite furnace atomiser. The higher sensitivity of the method means that solvent extraction or preconcentration procedures may be avoided; sample preparation and handling may be minimised.

However the graphite furnace atomiser is not recommended if;

(a). The sample is already in a liquid form and the concentration of the analyte permits a simple analysis by flame A.A.

(b). Certain refractory elements such as tungsten, tantalum or zirconium are to be determined. Some elements can not be easily atomised by graphite furnace and flame A.A. remains the best technique.

Furnace atomisation is a single measurement analytical technique, i.e. a fixed volume of sample is analysed at one time, in contrast to flame atomisation where the sample is continuously aspirated and many measurements can be taken during the aspiration period.

The function of the graphite furnace is to generate a population of free atoms so that atomic absorption can be measured. This is generally achieved in 3 stages.

1. The Desolvation (Drying) Stage.

This part of the programme plays an important role in the determination of the precision of analysis. The temperature and the time are chosen to give complete desolvation of the sample before the ash stage is reached. Generally, the temperature is set just below the boiling point of the solvent to ensure a sufficiently high rate of vapourisation of the solvent. If the temperature is too high the sample may splatter, resulting in loss of sample to the extremities of the furnace tube or onto the window of the atomiser.

The temperature programme for desolvation of samples containing 2 or more solvents, such as strong aqueous acid solutions or mixed organic solvents, may be adjusted to take account of the different boiling points of the solvents. The first temperature of the desolvation stage is programmed in relation to the lowest boiling constituent of the solvent. As the most volatile solvent is removed, the temperature may be increased to effect vapourisation of the less volatile solvent. The total dry time will usually be between 1 and 3 seconds.

2. The Ash Stage.

An ash or char stage may be necessary to further remove sample matrix components prior to the atomisation stage. Matrix components may vapourise or decompose on heating to give background absorption resulting from absorption by molecular species or light scattering from particulate matter. The time parameters in this stage are optimised for maximum removal of the sample matrix consistent with no loss of the analyte element. The ash temperature for organic matrices usually lies in the range 400-800^{o}C and the time is often comparable to the desolvation time.

It may not be possible to remove matrix components completely without also causing loss of analyte. In such cases, the background adsorption may overlap the atomic peak and simultaneous background correction is necessary.

The temperature-time parameters for this stage may be optimised using measurements of the absorption signal during the atomisation stage. The maximum temperature for ashing may be determined by increasing the ash temperature and measuring the background correction absorbance for the analyte. When loss of the analyte occurs during the ash stage observable reduction of the absorbance will occur. In some cases the analyte and background peaks can be separated more effectively by matrix modification.

3. The Atomisation Stage.

The atomisation temperature and the rate of heating of the atomiser from ash to atomise temperature (the ramp rate) affect the sensitivity of the analysis. Atomisation temperatures vary from element to element and the optimum atomisation temperature may be determined by increasing the programme temperature and measuring the peak atomic absorption. Generally the optimum temperature will be the lowest temperature giving the maximum absorption, since the lifetime of the graphite tube is prolonged at lower temperatures. The peak absorption does not show a continuous increase with atomisation temperature because the rate of loss of atomic vapour from the atomiser also increases with temperature. Loss of atomic vapour occurs by diffusion, convection and expansion of the gas in the atomiser.

The atomisation time is usually set as the minimum time required for complete vapourisation and removal of the analyte from the atomiser. This is the time required for the atomic absorption signal to return to the baseline.

Interferences.

The interference effects may be described as either physical or chemical in the mechanism by which they are produced.

Physical effects of viscosity and surface tension are less than for flame A.A., but nevertheless they can affect the reproducibility of sample dispensing. The main effect is in the degree to which the sample spreads inside the graphite tube. Another physical effect is that of the background which can normally be corrected for with the use of a suitably designed background corrector. For practical analysis, it is essential to use a background corrector for graphite furnace work. Chemical interference effects normally demand much more consideration in order to ensure that an accurate answer is obtained from an analysis. These interference's can be categorised as;

(a). Volatile component formation, where the analyte element is lost at a relatively low temperature, perhaps during a dry or ashing stage, without undergoing atomisation.

(b). Stable compound formation in which one or more relatively stable compounds of the analyte element are formed. More than 1 absorption peak could result because the appearance temperature will depend on the nature of the intermediate chemical form of the element.

Controlled chemical interference's effects can be used successfully in changing the appearance temperature and improving the sensitivity of the analyte, or changing the appearance temperature and magnitude of non-atomic absorption due to the matrix.

Development of Varian's Graphite Furnace Atomiser.

The first carbon rod atomiser to be produced commercially was developed and marketed by Varian in 1970. This device was largely patterned after the device

described by West and William's in 1969. The work head consisted of 2 water cooled terminal blocks which supported a single electrode. The original filament, often referred to as the "west" rod, contained a small indent in the centre of the rod where the samples were evaporated, ashed and atomised. Because the atoms were observed in an open region above the hot graphite this gave rise to several chemical interference's. At about the time that Varian's carbon rod atomiser made its first appearance, commercial graphite furnace atomisers were being developed in Europe and America. The most interesting of these was the heated graphite atomiser based on Massmann's work with the graphite tube. The heated tube concept employed in Massmann's design confined the atoms, and so increased their residence time in the optical path and decreased chemical interference problems. Next a miniature furnace approach was developed by changing the electrode design. This new design was called the "Mini-Massmann" rod. It consisted of a graphite rod 5mm in diameter with a transverse hole of 1.5mm diameter. But this design produced a furnace which could only accommodate volume of a maximum 2μL.

With the introduction of the Model 63 carbon rod atomiser in 1972, many of these problems which limited the use of "flameless" atomisers were eliminated. The next development in furnace atomisation, the CRA-90 carbon rod atomiser maintained the elegance and simplicity of the Model 63, but offered better power control, increased cooling capability to speed analyses, and temperature readout capability for operator convenience. Two types of furnace atomisers were then available - the tube and the cup. The tube furnace was generally more sensitive; the cup furnace allows the use of powdered or solid samples. To protect the incandescent graphite from excessive corrosion, there is an upward flow of inert gas which surrounds the heated graphite.

The next Varian development was the GTA-95, pyrolytic coated graphite atomiser, which offered many advantages over the CRA-90 which include;

(a). Analytical sensitivity.

(b). High sample volume capacity for improved detection limits and ease of sample introduction.

(c). Multi-step temperature programme to provide exact drying, ashing and atomisation temperatures.

(d). Ability to produce a second atomisation during one analysis in order to reduce memory or remove residual contaminants.

(e). Inert gas flow can be set at any time during the analytical programming to permit ashing or to enhance the atomisation signal.

(f). Simple routine preparation of standard additions and addition of chemical modifiers when coupled to the auto-sampler.

Pyrolytic Graphite Coatings.

The pyrolytic graphite coating on the graphite tube is a vital component of a successful analysis. The thickness of the coat is about 30μm. In the manufacturing process pyrolytic graphite is deposited on the substrate graphite from the vapour phase after thermal decompositon of a simple hydrocarbon such as methane under low pressure. As this deposition proceeds, a dense, dark layer of graphite carbon is built up over the substrate graphite. The crystals of the pyrolytic graphite lie virtually parallel to the surface and it is highly anisotropic, both electrically and thermally.

Advantages of a pyrolytic graphite coating would include;

(a). The coating is relatively impermeable to hot gases.

(b). It is more resistant to oxidation than normal graphite.

(c). It is unreactive chemically, and the tendency for some refractory elements to from carbides is significantly reduced. (Analytical methods for Graphite tube Atomisers (1982)).

Phifer (1995) used graphite furnace atomic absorption spectroscopy (G.F.A.A.S.) for the determination of chromium in medical foods. A fixed atomisation temperature of 2500°C for 5 seconds was selected. The preheating temperature was set at 1600°C for 3 seconds. Using these parameters a linear calibration curve was obtained from 5 to 25 ppb chromium and a detection limit of 0.24 ppb Cr(VI) was established.

Li and Shi (1997) also used G.F.A.A.S. for the analysis of trace Cr(VI) in water after preconcentration on a soluble membrane filter. This method was based on the formation of a chromium (VI)-salicylflurone (SAF)-tetra-decylpyridinium (TDP) ternary complex, which was then collected on a nitro-cellulose membrane filter. The complex and the membrane filter were then dissolved in concentrated H_2SO_4. Then using the following conditions the Cr(VI) concentration was determined using G.F.A.A.S.; inject volume of sample 20μL; measuring wavelength 359.3nm; slit 1.3nm; lamp current 7.5mA; drying temperature and time 60-100°C and 40s; ashing temperature and time 2700°C and 8s. The nitro-cellulose membrane filter was chosen because of its solubility in solvents such as concentrated H_2SO_4. H_2SO_4 was used because it was found that $0.2cm^3$ was sufficient to dissolve the nitro-cellulose membrane and the ternary complex in only half a minute. Using this method the determination of Cr(VI) less than $0.1\mu g\ dm^{-3}$ was possible.

1.9.(c). U.V. Spectroscopy.

Principle.

The electronic spectra of molecules are found in the wavelength range 1000-8000A° of the electromagnetic spectrum. The visible region to which the human eye is sensitive, corresponds to the range of wavelengths between 4000-8000A°. The U.V. region is subdivided into 2 spectral regions. The range between 2000A° and 4000A° is referred to as the near U.V. region. The unit of wavelength commonly used in visible or U.V. spectroscopy is the angstrom unit, A°, or the millimicron, mμ, equal to 10 A°. Sometimes the wavelength number, $v=1/\lambda$, is also used. The unit of wavenumber is the reciprocal centimetre, cm^{-1}.

Elementary Theory.

There are two classes of spectra, mainly emission and absorption spectra. An emission spectrum is obtained by analysing light emitted by a luminous source. An absorption spectrum is obtained by the spectroscopic analysis of the light transmitted by an absorbing medium, which is placed between the light source and the spectroscope. When a molecule absorbs radiation, its energy increases. This increase is equal to the energy of the photon as expressed by the relation

$$E = hv = hc / \lambda$$

where h is Planks constant, v and λ are the frequency and the wavelength respectively and c is the velocity of light. The change in energy may be in the electronic, vibrational or rotational energy of the molecule. Changes in electronic energy may involve relatively large quanta. Changes in vibrational energy involve smaller quantities of energy and changes in rotational energy involve quanta even smaller than those involved in vibrational energy. The electronic energy level of the molecule

under normal conditions is called its ground state and the higher energy levels represent the 1^{st} and 2^{nd} excited states respectively. For each electronic level there are the ground state and several possible excited vibrational states and similarly, for every vibrational level there are the ground and excited rotational levels (figure 1.9). The energies associated with electronic transitions are very large. $20000cm^{-1}$ is approximately 56K cal mol^{-1} and this energy is sufficient for the dissociation of many molecules. Since electronic transitions occur at even higher frequencies it becomes obvious that these transitions are accompanied by changes in the electronic distribution of molecules. If a molecule absorbs a small amount of energy from a source of radiation in the far infra-red region or the microwave region, only its rotational energy will change, no matter which vibrational or electronic state it is in. If the radiation source is of greater energy, say in the near infra-red region, then both the vibrational and rotation energies of the molecule will change. If the energy from the radiation is much greater, as in the case of U.V. light, changes in the electronic, vibrational and rotational energies will take place. Consequently, the U.V. spectrum of a molecule will be more complicated than its rotational or rotational-vibrational spectrum. There will be such a large number of closely spaced sub-levels as to make the UV spectra of polyatomic molecules, even in the gaseous state, appear only as broad adsorption band or band envelopes.

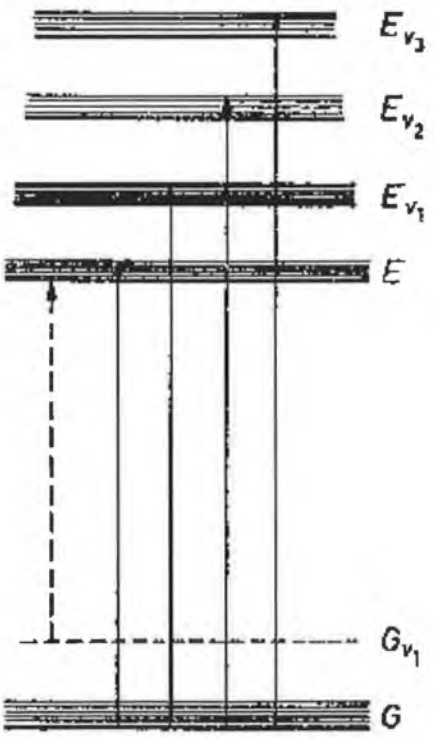


Figure 1.9. Energy levels for a polyatomic molecule and the origin of absorption electronic states; E, excited energy state; V_1, V_2, V_3, different vibrational states. The closely packed lines represent rotational levels.

In an electronic transition, the excited molecule may rotate to the ground state giving up its excess energy as heat or as fluorescent radiation of longer wavelength. Another possibility is for the excited molecule to undergo homolytic dissociation or ionisation. While the former process can take place by absorption or radiation in the 2000-8000A° range, the latter process usually takes place by absorption in the vacuum U.V.-region. Beyond the ionisation potential, there will be a region of continuous absorption.

Absorption Intensity.

Before the development of adequate theory, Beer and Lambert had proposed laws of light absorption. These laws are well known in the combined form as the Beer-Lambert law of light absorption, which states that the fraction of the incident light absorbs in proportion to the number of molecules in the path. This law is expressed as Absorption or Extinction or Optical Density = $\log_{10}$ Io / I = Ecl.

where E is the absorption coefficient, c is the concentration and I is the path length. If c is expressed in mole per litre and I in cm, then the absorption coefficient becomes a molar extinction coefficient. The absorption intensity is usually expressed in terms of E and the majority of the applications in spectroscopy are based on the above mentioned equation.

According to the Beer-Lambert law, the absorbance of a solution should remain constant as long as the product of the concentration and the path length is constant. But this is not always true. Quite often the molar extinction coefficient varies appreciably with the concentration of the solute. These deviations may be due to one of many possible causes; molecular association of the solute at higher concentration; ionisation of the solute in the case of acids, bases and salts; fluorescence of the solute etc.. It is now an accepted rule in spectroscopy that one should not assume the Beer-Lambert law to hold good for any substance without confirming it first. The absorption intensity of an electronic transition at any wavelength is governed by the probability of the transition and the size of the absorbing molecule. The absorption maximum of the band therefore corresponds to the most probable transition in that region of absorption. The extinction coefficient may be expressed as E = kPa where k is a constant of the order 10^{20}, P is the probability and a is the area of cross-section of the molecule.

Instrumentation.

The basic parts of any type of spectroscopic equipment are the radiation source, the detector and the detector output measuring instrument. In the U.V.-region and the visible region, the sources are incandescent lamps or discharge tubes. Prisms or gratings are used as monochromators. If prisms are used the prism material will vary from one region of electronic spectra to another. Calcium fluoride or lithium fluoride are used in the vacuum region while quartz prisms are used in the near U.V.-region. For the visible region glass prisms are employed. The eye, the photographic plate and the photoelectric cells are used as detectors for the visible region. The optical system of a typical photoelectric spectrophotometer is shown in its simple form in figure 1.10.

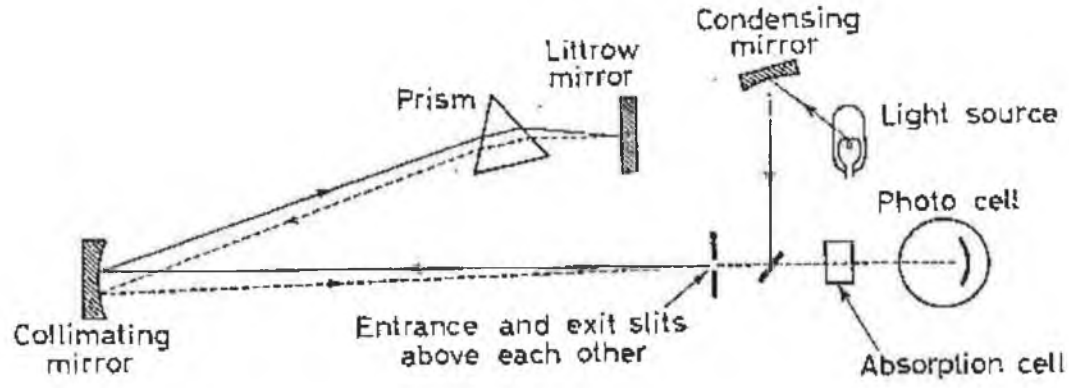


Figure 1.10. Optical system in a simple photoelectric spectrophotometer.

Selection Rules.

A brief summary of the important selection rules is outlined here. The 1st selection rule applies to all molecules of symmetry and deals with the parity-forbidden transitions (g - g or u - u). The 2nd rule is related to the multiplicity of states. According to this rule, singlet-triplet transitions should be forbidden. The 3rd rule

pretains to forbidden transitions arising from the symmetry of states (e.g. 260mμ band of benzene or the long wavelength bands of C=O, C=S and other chromophores). In addition to these three types of forbidden transitions, there are also other weak transitions which have low intensities.

Many forbidden transitions are observed with finite intensity in many molecules due to some intramolecular or intermolecular perturbations. Thus, singlet-triplet transitions occur with increased intensities in the presence of paramagnetic substances (NO or O_2) or in solvents which contain heavy atoms such as ethyl iodine. Vibrational interactions induce allowed character to forbidden transitions in some molecules. For example the forbidden band of benzene at 260mμ becomes weakly allowed due to the distortion of the D_{6h} symmetry of benzene by bending vibrations.

In the case of allowed bands, the symmetric vibrations of the molecules give rise to progressions in the excited state frequency starting from the zeroth vibrational level of the electronic ground state and the relative intensities give a measure of the change in molecular size and shape on excitation. In forbidden transitions which become allowed by a bending or some other vibration, the band origin is not found.

Franck and Condon have proposed an important rule for understanding the nature of electronic transitions. According to the Franck-Condon principle, transitions from the vibrational level of one electronic state to the vibrational level of another occur so rapidly that the position and velocities of the nuclei have no time to change, i.e., the nuclei remain fixed in position for the duration of the electronic rearrangement. The potential function of a vibrational mode of a molecule will depend on the electronic state of a molecule and the internuclear distance in the electronic state will therefore differ from the ground state value. In figure 1.11 the potential energy curves for upper and lower electronic states are shown for cases where the difference in two

states is zero, small or large. The Franck-Condon principle states that electronic transitions will take place only when the intermolecular distances are not significantly different in the two states and where the nuclei have little or no velocity.

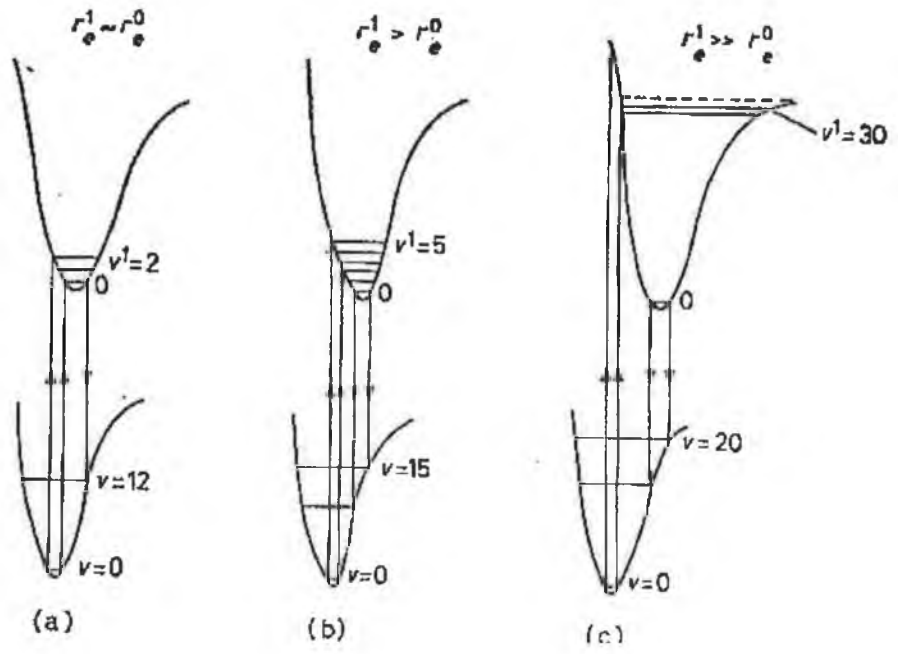


Figure 1.11. Diagram illustrating the Franck-Condon principle in three cases with increasing difference between the equilibrium distances in the electronically excited and ground states.

Cells and Solvents.

In recording the absorption spectra, two cells are used, one as the reference cell and the other as the sample cell. In the case of solution spectra, the reference cell is filled with the solvent and the sample cell with the solution. Such an arrangement compensates for solvent absorption and also for losses of radiation by scattering and reflection. Absorption cells of different dimensions made of glass or quartz are available. While quartz cells may be used in the entire near U.V.-visible region, glass

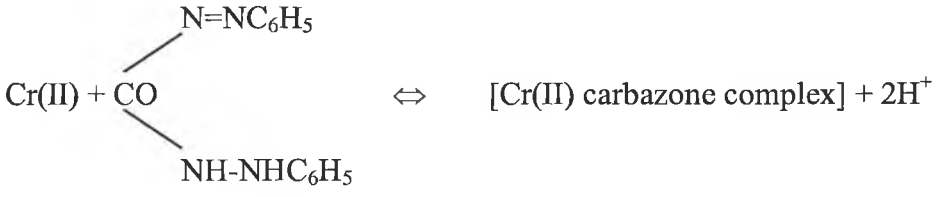
cells can be used only for the visible region. Cells of thickness anywhere from 0.1cm to 10cm are available, although 1cm cells are most generally used for everyday work. Spectra of gases are taken using enclosed cells, with an evacuated cell as a reference. Spectra of solids may be taken in the form of pellets. Pellet holders for the measurement of solids are commercially available. Most of the spectrophotometric studies are made in solution and the important factor in deciding the choice of solvent is that the solvent should not absorb in the same region as the solute. The solvents one can use for the near U.V.-and visible region are; water, alcohol, acetonitrile, chloroform, dioxane, hexane, iso-octane and cyclohexane. Purified hexane and heptane may be used in the vacuum UV region. (Ultra-Violet and Visible Spectroscopy (1967)).

Reaction of Chromate and Diphenylcarbazide.

The reaction between chromate with 1,5-diphenylcarbazide (DPC) has been widely used for the spectrophotometric determination of chromium since 1900. A very striking feature of this reaction is that the chromium must be present in its hexavalent state and the reaction medium should be sufficiently acidic. When the solution is alkaline, diphenylcarbazide does not react with chromate. This indicates that the reaction is primarily a redox one. However, since other oxidizing agents like nitrate, permanganate, ceric salts, iodine etc. fail to produce the characteristic violet colour with carbazide, it appears probable that the colour produced with chromate is due to some secondary complex formation. Oxidation plays a primary role in the complete process leading to colour formation.

Diphenylcarbazide when oxidized with chromate may give rise to carbazone or to its still higher oxidation product carbadiazone or the carbazide molecule itself may be

completely ruptured at the C=O linkage. The chromate molecule will be simultaneously reduced during the oxidation process and chromium in a lower state of valence is expected, which may act as a cation in further complex formation. This is an important factor since both carbazide and carbazone (but not carbadiazone) are known to be typical agents, which can combine with a large number of metal ions to produce intensely coloured inner complex salts. Reduction of chromate (Cr^{6+}) in acid medium leads generally to the trivalent (Cr^{3+}) state. But chromic acid is known to be non-reactive towards either carbazide or carbazone. The conclusion drawn from this is that chromium in the bivalent state (Cr^{2+}) is the ion responsible for the colour reaction. (Bose (1954).



Diphenylcarbazone

Presence of a Single Coloured Complex.

In the case of a mixture of several coloured complexes, the absorption spectra of solutions containing the reactants in different molar proportions in which they are expected to react would not show a constant wavelength of maximum adsorption. Therefore, mixtures of chromate-carbazide solutions were prepared in the molar ratios of 1:1, 1:2 and 1:3 keeping the chromium concentration constant and the spectral curves were obtained. In all cases, the wavelength of maximum absorption remained unchanged, excluding the possibility of formation of several complexes. (Bose (1954)).

Nature of the Complex Formed.

The coloured Cr^{2+} complex may either be a monovalent cation or an inner complex salt. To establish the nature of the complex, whether electrolytic or not, migration studies as well as solubility determinations in non-polar solvents were carried out.

Transport Experiments.

Almost saturated solutions of carbazide in alcohol were mixed with aqueous acidified chromate and this mixture was subjected to migration study. Even after 4 hours of electric current, the red colour failed to move within the limbs of the apparatus showing that the compound in non-electrolytic in nature.

Solubility Studies.

The solubility of a compound in various non-polar solvents usually indicates its inner complex nature. The reagent carbazide dissolved in non-polar solvents was added to an acidified non-polar solution and the mixture was shaken in a stoppered test tube and allowed to settle. It was found that the nature of the acid used to acidify the reaction mixture had a profound effect on the partition of the coloured product between the 2 phases. In organic acid (AcOH) medium and with solvents C_6H_6 and $CHCl_3$ the coloured product was found to distribute itself between the 2 phases, the distribution being greatly in favour of the organic solvent, and with cyclohexanol, the aqueous layer was completely colourless. However, when mineral acids such as sulphuric acid (H_2SO_4) were used the organic layer was almost colourless except in the case of cyclohexanol. Even then, the colour in the cyclohexanol layer is much less in contrast to that produced in organic acid medium. Thus, the reaction of carbazide

or carbazone may be considered to be in conformity with their well-known property of forming inner-complex salts with other bivalent metals. (Bose (1954)).

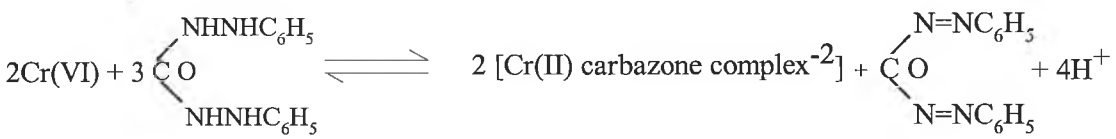
Stoichiometry of the Reaction.

Bose (1954) investigated the stoichiometry of the reaction of chromate and carbazide and also that of the reaction of chromate and carbazone and chromous and carbazone.

Chromate-Carbazide system.

The reagent carbazide is colourless and though K_2CrO_4 solutions are slightly coloured, at the dilutions Bose employed, they were for all practical purposes colourless. To determine the maximum composition of carbazide the observed absorbances (A) were directly plotted against composition for equimolecular chromate-carbazide mixtures at 550mμ of concentration (i) 4.10 x 10^{-5}M, (ii) 3.10 x 10^{-5}M and (iii) 2.10 x 10^{-5}M. From these curves it was concluded that the maximum composition corresponds to a chromate: carbazide ratio equal to 2:3. The maximum composition proved to be the same for different initial concentrations of the equimolecular mixtures. The maximum composition was found to be independent of the wavelength of light, which again confirms the existence of a single complex in the system.

Equation (1).

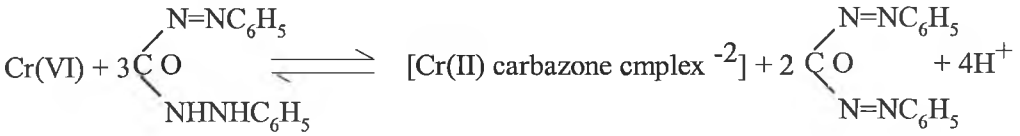


diphenylcarbazide diphenylcarbadiazone

Chromate-Carbazone System.

Carbazone is coloured but again at the dilutions employed it's contribution to the absorbance could be neglected and again absorbance values were directly plotted against composition. From this it was found that the maximum composition of chromate: carbazone ratio equals 1:3 for the different initial concentrations of the equimolecular mixtures.

Equation (2).

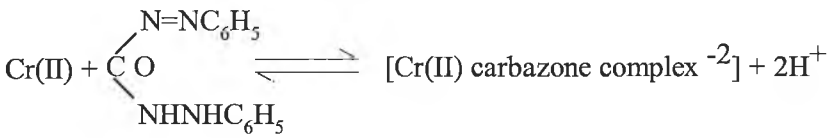


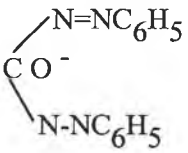
diphenylcarbazide diphenylcarbadiazone

Chromous-Carbazone System.

Here the absorbance of a blank acidified carbazone-water mixture was first determined and these values were subtracted from the absorbance values of the mixture. The corrected absorbance values were then plotted against the composition of the mixture. The maximum composition in this case was then found to correspond to the chromous-carbazone ratio equal to 1:1 and again it is independent of the concentration.

Equation 3.



where carbazone $^{-2}$ = 

From the above-proposed mechanisms it can be seen that the same coloured complex is produced in all 3 reactions, and that there is a greater sensitivity of diphenylcarbazide as compared to carbazone for hexavalent chromium. The determination of the maximum composition revealed that for the same concentration of chromate, the amount of carbazone required is double that of carbazide required and hence the superiority of the carbazide reagent.

The primary function of chromate as an oxidizing agent in equation (1) and (2) is stepwise, first from carbazide to carbazone and then from carbazone to carbadiazone which is one of the products of equation (1) and (2). From the composition-absorbance curves for equation (1) and (2) it appears that after the maximum composition, any excess of chromate will oxidize Cr^{+2} to Cr^{+3} and the carbazone to carbadiazone or its higher oxidation product. But for equation (3) the excess Cr^{+2} will simply reduce the carbazone to carbazide, which is non-reactive towards bivalent chromium. (Bose(1954)).

Heringer Donmez and Kellenberger (1989) used two variations of DPC analysis for the determination of hexavalent chromium. The first method required the acidification of the sample prior to the introduction of the DPC, while the second method called for the addition of the DPC prior to sample acidification. The second method proved to be the superior in this work because knowing that acid conditions enhance the reaction of Cr(VI) with organic and other reductants, it is suspected that some Cr(VI) might be lost in certain samples. Therefore, by adding the DPC prior to acidification at least the Cr(VI) has a chance of reacting with the indicator (DPC) which will be present with other potential reductants. Indicator colour intensity was measured at 540nm wavelength on a U.V.-visible spectrophotometer. Before any

samples were actually analysed, a potential source of error was noted in the preparation of standards, blanks and controls for the DPC colourimetric measurements. Even though none of the blanks were coloured in the range typical of the indicator several did have a slight yellow or brown tint. When compared to distilled water, these yellowish tinted solutions gave small positive absorbances, and the amount of absorbance was not necessarily proportional to the intensity of the yellow or brown colour. Therefore without a more appropriate blank (i.e. samples without indicator added) the background error produced a coloured or turbid sample solution which would automatically be calculated as hexavalent chromium. It was also found that iron salts and humic acids produced slightly coloured solutions which absorbed slightly in the DPC measurement range. But these interferences were easily corrected by proper use of blank and sample controls, which allowed the sample solution to be calibrated as zero absorbance when measured without indicator additions. They also used chromium soil leaching to determine the chromium content of samples with atomic absorption and the second DPC method from above. Since the atomic absorption determination represents total chromium in the leachate, not just hexavalent chromium which was represented by the DPC results a clear contradiction in atomic absorption and the DPC results was observed. Theoretically, the DPC results should never exceed the atomic absorption results for the same samples. But in this case it was found that the DPC results greatly exceeded the atomic absorption results. The conclusion drawn form these results was that although DPC works well in many relatively clean solutions, a complex matrix such as soil leachate poses additional problems. Simple correction commonly used in spectrophotometric analysis are not sufficient to compensate for the highly variable and intense interferences possible in such a matrix.

Hoshi and Konuma (1998) preconcentrated the Cr(VI) –DPC complex on a column of chitin in the presence of dodecyl sulphate as counter-ion. They then eluted the Cr(VI)-DPC complex from the column using $1cm^3$ of 7:3 v/v methanol-1M acetic acid mixture as eluent. The mixture of methanol with 1M acetic acid was selected as eluent because it gave the best reproducibility compared with those of acetone or N,N-dimethylformamide (DMF) from previous works, and 7:3 v/v methanol-1M acetic acid because the absorbance of the eluent was not reproducible with over 90% of methanol concentration in the eluent. It was also found that the chromium complex collected on the chitin was readily eluted with $1cm^3$ of the eluent within 1 minute and that the absorbance of the eluent obtained was constant for at least 60 minutes. The calibration curve obtained was linear over the range 0.05-0.6μg of Cr(VI) in $1cm^3$ of eluent. The reducing agents, some metal ions in the presence of EDTA and oxoanions did not interfere in concentrations up to 100-1000 times that of Cr(VI). The common anions and EDTA did not interfere in concentrations up to 5000-10000 times that of Cr(VI). Here the reagent blank was considered as negligible and all measurements were made against distilled water at 540nm.

Allen (1958) also used 1,5-DPC in conjunction with atomic absorption spectrophotometry for the micro determination of chromium. Most investigators of this reaction found that the maximum absorbance occurred at 540mμ. However, Allen found a slightly higher value of 546mμ for his work. The absorbance was 1% higher at 546mμ than at 540mμ. He also found that the molar absorbancy index was independent of pH in the range 1.02 to 2.43. However, a significantly lower value was found at pH 0.54, and at pH 3.28 the formation of the coloured reaction product is slow. (At higher pH the reaction is still slower). The reaction in ordinary distilled

water showed no significant difference from those in which triple-distilled water was used. The age of the 1,5-DPC solution was found to effect the absorbance. The originally colourless 1,5-DPC solution also required an amber colour on standing. Urone (1950) found that the loss of sensitivity is proportional to the amount of discolouration. The colour of the stock solution therefore provides a convenient criterion for its reliability. As long as the stock solution remains colourless it may be used, but it should be discarded when appreciable colour develops. The concentration of the DPC and the order of mixing of the reagents was also investigated by Allen. A concentration of 0.5×10^{-3}M and 1.0×10^{-3}M gave almost identical results. At a much lower concentration of 0.1×10^{-3}M the molar absorbance index was substancially lower. The 6 possible orders of addition of 1,5-DPC solution, sulphuric acid solution and potassium hydrogen chromate solution were tested. From the results obtained Allen concluded that the Cr(VI) and 1,5-DPC should not come into contact before acidification, but otherwise the order of mixing made practically no difference. This result, however, did not agree with that of Heringer Donmez and Kallenberger, Bose or Babko and Palii.

Bartlett and James (1979) used the DPC-chromate reaction for the analysis of chromium in soils. They found that of the 150 fresh field soils checked all but 15 produced positive Cr(VI) tests. Of these 15 negative ones, all but 2 had pH's less than 6. These 2 had near neutral pH's and were found to be highly reduced. Overall the soils were found to oxidise an average of 0.2μmol Cr / g of soil. It was found that the amount of chromium oxidised increased rapidly during the first 24 hour period and then decreased slowly, and that considerably more chromium was formed in moist samples than in dried and rewet samples. Therefore this study showed that a large

proportion of the Cr(VI) may be reduced by the soil if it was not leached out within a few weeks after formation. But these results also indicate that significant amounts of the chromium oxidized by the soil may remain there for a rather long period under favourable conditions, certainly long enough for leaching to occur.

Rubel and Terytze (1999) also used the DPC-chromate reaction for the analysis of chromium in soils. Measurements were obtained at 550nm against a reference cell filled with distilled water. But to account for coloured soil solutions they treated another $10cm^3$ of the same soil filtrate in the same manner, except this time no DPC was added, only acetone. The absorbance value obtained here was subsequently substracted from that obtained for the same soil filtrate with DPC. For analysis, soils of different types and with different geogenic and anthropogenic chromium concentrations were used. The highest extractable Cr(VI) load was found in the anthropogenic contaminated soils from a former electroplating company site, where 18% of the total chromium extracted was found to be Cr(VI). The method used here was found to be most suitable for a concentration range of approximately 0.2–25 mg/kg soluble Cr(VI) in soils.

1.9.(d). High Performance Liquid Chromatography.

Principle.

High performance liquid chromatography (H.P.L.C.) is a technique that has arisen from the application to liquid chromatography (L.C.) of theories and instrumentation that were originally developed for gas chromatography (G.C.). In analytical H.P.L.C. the mobile phase is normally pumped through the column at a rate of 1-5cm^3 min^{-1}. If the composition of the mobile phase is constant, the method is called 'isocratic' elution. Alternatively the composition of the mobile phase can be made to change in a predetermined way during the separation, which is a technique called 'gradient' elution. Gradient elution is used in situations similar to those used for temperature programming in G.C., and is necessary when the range of retention times of solutes on the column is so large that they can not be eluted in a reasonable time using a single or solvent mixture. In absorption chromatography, for instance, non-polar solvents are absorbed relatively weakly and should be eluted with a non-polar solvent, whereas polar solvents are absorbed more strongly and require a more polar solvent. If the sample contains a wide range of polarities, the separation could be done by changing the polarity of the solvent mixture during the separation. In other cases it may be necessary to use gradient elution where other properties of the solvent (e.g. pH or ionic strength) are changed.

As in other forms of chromatography, the time taken for the solute to pass through the chromatographic system (the retention time) is a characteristic of the solute for a particular set of conditions. However, to use retention data on their own for the identification of unknown solutes would be rather like trying to identify an unknown organic compound by simply measuring its melting point or boiling point. Many different solutes will have the same retention times for a particular set of conditions.

Chromatography is an excellent method for the separation of mixtures but it does not provide the detail necessary for the clear identification of the separated compounds. Such detail is provided by spectrometric techniques. Some detectors can record and store the U.V. spectra of solutions as they emerge from the column. The only restriction on H.P.L.C. for analysis is that the sample must dissolve in a solvent. Thus, H.P.L.C. is suitable for macromolecules, inorganic or other ionic species, labile natural products, pharmaceutical compounds and biochemicals.

An H.P.L.C. requires a high pressure pump and a supply of mobile phase, a column containing a high efficiency stationary phase, an injection unit for introducing samples onto the column, an on line detector and some method of displaying the detector signal (figure 1.12). The simplest reservoir is a $1dm^3$ glass bottle with a cap drilled to take a 1/8 inch diameter PTFE tube to carry the mobile phase from the reservoir to the pump. The liquid entering the pump should not contain any dust or other particulate matter, as this can interfere with the pumping action and can cause damage if it gets into the seals of valves. Such material can also collect on top of the column, causing irregular behaviour or maybe even blockages. The mobile phase is, therefore filtered before it enters the pump. This can be done using a stainless steel filter element that is a push fit onto the end of the PTFE tube in the reservoir, or alternatively an in-line filter can be used. The pore size is normally 2μm.

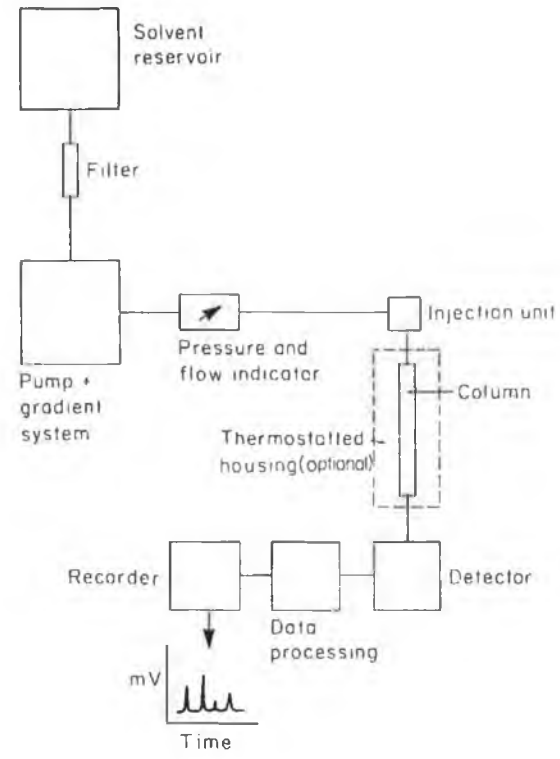


Figure 1.12. Block diagram of a high performance liquid chromatograph.

The function of the pump in H.P.L.C. is to pass mobile phase through the column at a controlled flow rate. One class of pump (constant pressure pump) does this by applying a constant pressure to the mobile phase; the flow rate through the column then being determined by the flow resistance of the column and any other restrictions between the pump and the detector outlet. Another type (constant flow pump) generates a flow of liquid, so that the pressure developed depends on the flow resistance. The flow resistance of the system may change with time, owing to swelling or settling of the pump packing, small changes in temperature, or build up of foreign particle matter from samples, pump or injector. If a constant pressure pump is used the flow rate will change if the flow resistance changes, but for constant flow pumps changes in resistence are compensated for by a change of pressure. Since flow changes are undesirable, as they will cause retention data to lack precision and may cause an erratic baseline on the recorder. It is advisable not to use constant pressure

pumps in H.P.L.C. instruments. However, they are suitable for packing columns where small changes in flow do not matter.

Column inlet pressures in H.P.L.C. can be as much as 200 times atmospheric pressure, but most of the work in analytical H.P.L.C. is done using pressure between about 25 and 100 bar. The pressure developed will depend on the length of the column, the particle size of the stationary phase, and the velocity and flow rate of the mobile phase. Because liquids are not very compressible there is not much energy stored in them at high pressure, and the pressures used in H.P.L.C. do not represent a hazard. Many commercial H.P.L.C. instruments provide a forced air oven, which will control temperature with a stability of typically 0.1°C from ambient temperature to 100°C. Because of the use of flammable solvents, safety considerations are important, so the ovens are usually provided with a facility for nitrogen purging, and are designed to prevent a build up of solvent vapour in the event of a leak. If temperature control is used, it is important that the sample and the mobile phase are at the right temperature before being introduced to the column, so the mobile phase is normally passed through a preheating coil housed in the oven before it reaches the injection point.

Commercial chromatographs use values for sample injection. Although they are expensive, they are easy to use, give good precisions, and are easily adapted for automatic injection. With these devices, the sample is first transferred at atmospheric pressure from a syringe into a sample loop. Turning the valve from load to inject position connects the sample loop into the high pressure mobile phase stream, whereby the contents of the sample loop are transferred onto the column. A variety of loop volumes are available, commercially 10-50μL. For smaller volumes than this, the loop is an engraved slot in the body of the valve.

The columns most commonly used at the moment are made with 316 grade stainless steel. Other materials used for columns include glass, glass lined steel tube and polyethene or other inert plastics. The inside of the stainless steel tube should be as smooth as possible, so the tubes are precision drilled or electropolished after manufacture. At the top of the column, there is a distributor for directing the injected sample to the centre of the column and then a stainless steel gauze or frit on top of the packing. At the lower end there is another frit, and the end of the packing, and then, for 4.6mm type, a reducing union and a short length of 0.25mm internal diameter tubing to connect the column to the detector.

The function of the detector in H.P.L.C. is to monitor the mobile phase coming from the column. The output of the detector is an electrical signal that is proportional to some property of the mobile phase and/or the solutes. If this property is possessed essentially by the solute, such as absorption of U.V./visible radiation or electrochemical activity, the detector is called a property detector. The characteristics that are required for an ideal detector are sensitivity, linearity, universal or selective response, predictable response, unaffected by change in conditions, low dead volume, non-destructive and cheap, reliable and easy to use. Sensitivity is the ratio of output to input, so that a large detector signal for a small amount of solute is produced. A linear detector will have a response that is directly proportional to the amount or concentration of solute. A universal detector will detect anything in the sample, whereas a selective detector will detect only certain components. Dead volume in a detector will add to the extra-column dispersions, so it must be kept to a minimum. This includes the cell volume of the detector and for spectrometric detectors a reduction in the cell volume is likely to lead to a loss of sensitivity. UV absorbance detectors are by far the most popular detectors in H.P.L.C.. The principle is that the

mobile phase from the column is passed through a small flow cell held in the radiation beam of a U.V./visible photometric or spectrophotometric detectors. These detectors are selective in that they will detect only absorbed UV (or visible) radiation. The mobile phase that is used should absorb little or no radiation. Absorbance of radiation by solutes as a function of concentration is described by the Beer-Lambert Law as A = Ecl (section 1.9.(c)., p 60). Strictly this law applies only to monochromatic radiation. However, the detector system does not provide truly monochromatic radiation but rather a narrow band of wavelengths centred around the selected wavelength. Both fixed and variable wavelength U.V. visible detectors are available. The variable types use a deuterium lamp and/or tungsten filament lamp as the radiation source, and can operate from 254nm to 280nm, but other wavelengths are possible. Other less common detectors are;

(a). The Photodiode Array Detectors (PDA), where polychromatic radiation is passed through the sample and then is focused onto the entrance slit of a monochromator, which passes a narrow band of wavelengths to the detector.

(b). Fluorescence Detectors, where many compounds are capable of absorbing U.V. radiation and subsequently emitting radiation of a longer wavelength, either instantly (fluorescence) or after a time delay (phosphorescence). Usually, the fraction of the absorbed energy that is re-emitted is quite low, but for a few compounds values of 0.1-1.0 are obtained, and such compounds are suitable for fluorescence detection.

(c). Electrochemical Detectors, which measure either the conductance of the eluent or the current associated with the oxidation or reduction of solutes. To be capable of detection using the first method the solute must be ionic, and using the second method the solute must be relatively easy to oxidise or reduce.

(d). Amperometric Detectors, which oxidise or reduce only a small quantity of the solute (less than 1%), so the currents observed are very low (nanoamps). Such currents are not difficult to use using modern amplifiers and the detector has a high sensitivity.

(e). Coulometric Detectors, are small multielectrode devices that can use up to 4 porous graphite working electrodes. The column eluent flows through these electrodes rather then over them and with proper choice of potential the detector reacts with all of the electroactive solute passing through it.

(f). Refractive Index Detectors, sense the difference in refractive index between the column eluent and the reference stream of pure mobile phase. Any solvent can be detected as long as there is a difference in refractive index between the solute and the mobile phase.

The relative distribution of a solute between two phases is determined by the interactions of a solute species with each phase. The relative strengths of these interactions are determined by the variety and the strengths of the intermolecular forces that are present, or, in more general terms, by the polarity of the sample and that of the stationary phase. Intermolecular forces may be caused by a solute molecule having a dipole moment, whereby it can interact selectively with other dipoles, or if a molecule is a good proton donor or acceptor it can interact with other such molecules by hydrogen bonding. Molecules can also interact via much weaker dispersion forces, which rely on a given molecule being polarised by another molecule.

Polarity is a term that is used in chromatography as an index of the ability of compounds to interact with one another in these various ways. It is applied very

freely to solutes, stationary phase and mobile phase. The more polar a molecule, the more strongly it can interact with other molecules through the mechanisms above. If the polarity of stationary and mobile phase are similar then it is likely that the interaction of solutes with each phase may also be similar, leading to poor separation. Thus, for hydrocarbon-type (non-polar) stationary phase we need a more polar mobile phase, whereas unmodified silica, which is highly polar needs a mobile phase with relatively low polarity. If the separation of solutes that are chemically very similar is required, then stationary phases that are chemically very similar to the solutes should be chosen. The retention of solutes is usually altered by changing mobile phase polarity. (Lindsay (1992)).

Padarauskas and Judzentiene (1998) determined the amount of chromate in a sample by precolumn complexation with 1,5-diphenylcarbazide. The diphenylcarbazide-chromate complex was then preconcentrated onto a C_{18} column. Preliminary investigations showed that an optical separation resulted from tests made with mobile phase containing 20% acetonitrile and 6×10^{-3} mol l^{-1} H_2SO_4. Under these conditions they found that 6 minutes was sufficient for the elution of the analyte. Minimum detectable concentrations using direct injection of 100µl and photometric detection at 546nm is approximately 6 ng ml^{-1} Cr(VI). On optimizing the preconcentration procedure it was found that a flow rate of 5 cm^3 min^{-1} was considered optimal, that the critical volume for the concentrator column was approximately 100cm^3 and also that 2×10^{-4} mol l^{-1} diphenylcarbazide concentration was sufficient. It was also found that most common cations and anions did not interfere with the Cr(VI) enrichment. A linear relationship between peak area and concentration was obtained in the 0.05-2.5 ng cm^{-3}.

Pobozy and Wojasinska (1996) determined the amount of chromate in a sample by post column complexation with 1,5-diphenylcarbazide, without retention of Cr(III) on the H.P.L.C. column. Here an optimum mobile phase was found to be a 4mM phthalate solution of pH 3.5. The obtained peak shape was also found to be affected by the pH of the injected sample solution and a pH of 3.0 was found to be the most favourable. Under these conditions a linear relationship between peak height and concentration in injected samples were obtained up to 2 mg dm^{-3} for Cr(VI) and up to 5 mg dm^{-3} for Cr(III). They also determined the amount of Cr(III) in a sample by post column complexation with DCTA (disodium salt of 1,2-diaminecyclohexane-N,N,N',N'-tetraacetic acid). Here an optimum mobile phase of 2mM phthalate pH 3.5 was obtained. The calibration plots which ranged from 10 to 100 ng cm^{-3} for Cr(III) and from 5 to 50 ng cm^{-3} for Cr(VI) were both linear.

Comparison of the detection limits of post column complexation and precolumn complexation with 1,5-diphenylcarbazide shows that the detection limits reported for the post column complexation are greater than those of the precolumn complexation. Thus, the precolumn complexation of chromate with 1,5-diphenylcarbazide is more sensitive than the post column complexation.

Saverwyns and Van Hecke (1999) investigated the efficiency of a commercially available microbore anion exchange column for chromium speciation with detection by ICP-MS and hyphenation with micro concentric nebulization. They found that 60mM nitric acid at a flow rate of 80µl min^{-1} was optimum for the mobile phase. At higher concentrations of mobile phase or at higher flow rate, no baseline separation was obtained. At lower flow rate, Cr(VI) eluted later, leading to a broader peak, and hence to a decrease of the sensitivity for this compound. Independent investigations

concerning the effect of the pH of the mobile phase showed that the pH had no significant influence on the separation, but must be kept between 1 and 7. At a pH less than 1, the CrO_4^{2-} appears as H_2CrO_4, eluting together with Cr(III) in the void volume. At a pH higher than 7, Cr(III) will precipitate. A loop size of 20µl was chosen as a compromise between a good sensitivity and baseline separation. The most predominant spectral interferences were expected for real samples containing chloride and/or carbonate ions. It was found that carbon did interfere with Cr(VI) determination, where ^{12}C concentration (m/z = 52) as low as 10 mg dm^{-3} caused interferences due to co-elution of HCO^{3-} with Cr(VI), and ^{13}C concentrations (at m/z = 53) as low as 250 mg dm^{-3} caused interferences. At m/z = 53 the determination of Cr(III) was also found to be hampered by ^{37}Cl occurring from a concentration of 10 mg l^{-1}. At m/z = 53 however no Cl-based spectral interferences could be detected.

Powell and Boomer (1995) used high pressure liquid chromatography (H.P.L.C.) combined with direct injection nebulization (D.I.N.) and inductively coupled plasma mass spectroscopy (I.C.P.-M.S.) to determine the chromium speciation in samples. They found that the eluent flow rate through the column was a major factor affecting analyte separation, retention time, and signal strength. The lower the flow rate, the broader the peak widths, and hence, greater separation. As well, peak height decreases at a lower flow rate. This seemed to indicate a limitation on sensitivity by improving separation. However, using peak area measurements compensated for loss in peak height, and for the most part, detection limits were retained. Eluent strength was another leading factor, which was found to contribute to the overall performance of the chromatographic system. The acid strength was used to determine how long the analyte was retained since trivalent chromium is cationic under acidic conditions.

This was found to affect retention time and peak separation of the resulting chromatogram, which showed differences as very narrow peak widths for the highest acid concentration to extremely broad peak widths for the lowest acid concentration. From these results, the eluent strength chosen as optimum was 0.25% HNO_3. Experiments in previous works showed that an increase in sensitivity may be gained by using a larger sample loop. The smaller loop sizes showed good separation, but poor signal. The larger loop sizes showed narrowed peak widths with a distorted signal, which was caused by analyte overloading of the column. From these results, the optimum sample loop size chosen was 10µl. The detection limits obtained here for Cr^{52} and Cr^{53} for Cr(III) were 180 and 60 ng dm^{-3} respectively. Using Cr^{53}, the Cr(VI) detection limit was 180 ng dm^{-3}, and total chromium detection limit was determined to be 30ng dm^{-3}.

1.10. Reaction of Chromate by Ascorbic Acid.

L-ascorbic acid (Vitamin C), found naturally in plants and animals, in not biologically synthesised by humans. The carcinogenicity of chromium (VI) is thought to be related to the oxidation of various cellular constituents. Ascorbic acid, being a constituent of the cell and a good reductant, may therefore function as an antichrome agent in vivo against chromate poisoning.

L-ascorbic acid (H_2A) is very widely known and used for its reducing properties. Among the many reactions studied are those involving its efficient reduction of many transition-metal ions and complexes by outer- and inner-sphere mechanisms. It has also been shown that L-ascorbic acid is oxidised by the potentially carcinogenic chromium (VI) ion at the body's physiological pH of 7.40, as well as at lower pH. At physiological conditions most Cr(VI) exists as tetrahedral, CrO_4^{2-}, and pseudo-tetrahedral, $HCrO_4^-$ ions. These ions can easily transverse the cell membrane via pre-existing anions, eg. the sulphate transport system.

Many small molecules found within the cell are redox active and should be able to reduce chromium (VI) based on their redox potentials. These compounds may be important to the cellular metabolism of chromium (VI). Extrapolation to pH 7.40 using the Nernest equation gives a redox potential of +0.52V for the chromium (VI) – chromium (III) half-reaction.

$$CrO_4^{2-} + 4H_2O + 3e^- \rightarrow Cr(OH)_3 + 5\ OH^- \qquad (1)$$

However, most studies of chromium (VI) oxidations of small molecules have been carried out under acidic conditions, but some have been carried out under conditions of physiological pH. These studies showed that the chromate reduction by small molecules is kinetically controlled, rather than thermodynamically controlled, and that ascorbic acid reacts at a significant rate at pH 7.40.

Stoichiometry and Mechanism of the Reaction.

The accepted stoichiometry of the reaction of chromate with ascorbic acid is

3 moles of ascorbic acid react with 2 moles of chromate, each ascorbate transfers 2 electrons and overall 3 moles of dehydroascorbic acid and 2 moles of trivalent chromium are formed.

$$3HA^- + 2[HCrO_4^-] + 3H_2O \rightarrow 3A' + 2Cr^{3+} + 11OH^- \qquad (2)$$

The break point occurs at $[H_2A] / [CrO_4^{2-}] = 1.5$

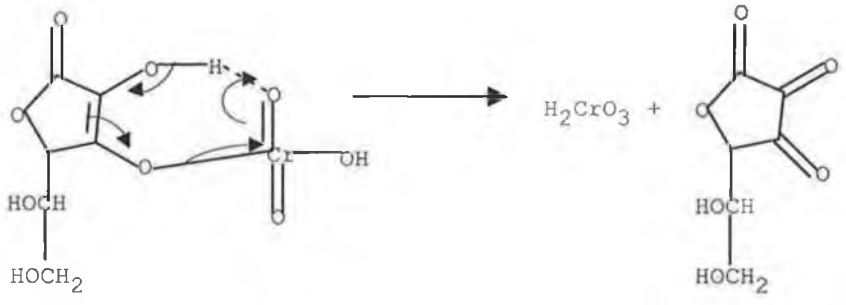
where H_2A = ascorbic acid

A' = dehydroascorbic acid

Dixon and Sadler (1993), established this break point by varying the $[H_2A]$:$[CrO_4^{2-}]$ ratio from 1:1 to 1:10 at 25°C, pH 7.40 (Tris-HCl buffer). Above the ratio of 1:1.5 little or no difference was observed for the absorbance of the product of the reaction.

A possible mechanism for the reaction involves the formation of a chromate-ascorbate ester intermediate, which then undergoes a unimolecular redox reaction. It is the formation of this chromate-ascorbate ester intermediate, which provides a low energy pathway for the transfer of electrons via an inner sphere mechanism.

The chromate-ascorbate ester intermediate produced may be of the form shown on the left of the following diagram and can undergo electron transfer as indicated.



Ester formation is favoured at lower pH, as the undissociated ascorbic acid, H_2A, can lose a proton to an OH of $[HCrO_4]^-$ thereby increasing the liability of the Cr-O bond.

This ensures the easy loss of a molecule of water. This proposed intermediate could decompose via a second route without the transfer of electrons. It may revert to the original reactants, and it is this pathway which is described by K_r in equation (4).

$$H_2A + [HCrO_4]^- \underset{k_r}{\overset{k_f}{\longleftrightarrow}} \text{products} \qquad (4)$$

It is the formation of this chromate-ascorbate ester intermediate that is thought to be the rate determining step of the reaction. The product of this reaction is the chromate-ascorbate ester intermediate and the resulting rate expression is

$$k_{obs} = k_f [\text{ascorbic acid}]_t + k_r \qquad (5)$$

where k_r and k_f are the slope and the intercept respectively from the graph of k_{obs} verses [ascorbic acid]. Since the forward reaction, (k_f), gives much larger values than the reverse reaction, (k_r), it can be assumed that the reverse reaction is insignificant as a reaction pathway. The equilibrium constant, (K_e), can be estimated from k_f and k_r for the reaction since

$$k_e = k_f / k_r \qquad (6)$$

Ascorbic Acid and pH Dependence of the Reaction.

Kinetic runs were carried out at 25°C with the ascorbic acid concentration ranging from 0.002 to 0.040 mol dm^{-3} over the pH range 4.60 to 7.40. The psuedo-first-order rate constants increased with increasing ascorbic acid concentration and decreased as the pH increased at a fixed ascorbic acid concentration. Plots of k_{obs} v's [ascorbic acid] were linear with small intercepts, which decreased with increasing pH. These results indicated that the reaction is first order with respect to the ascorbic acid concentration and also that there is a second step independent of ascorbic acid concentration making some minor contribution, especially at lower pH. (Dixon and Sadler (1993)).

O'Brien and Woodbridge (1997), also found that the pseudo-first-order rate constants were directly proportional to the concentration of ascorbic acid and that the rate increased with ascorbic acid concentration. A plot of k_{obs} v's ascorbic acid concentration proved to be linear with slope close to unity, again confirming the reaction as first order with respect to ascorbic acid concentration.

According to Dixon and Sadler (1993), the pH dependence of the rate constants suggests that $[HCrO_4]^-$, H_2A and HA^- are the reactive species in the rate-determining step producing the chromate-ascorbate ester intermediate. The very small concentration of dichromate was such that the amount of the dimeric product, $[Cr_2O_7]^{2-}$, was negligible at all pH values. Below pH 5, H_2A is the dominant form of the ascorbic acid whereas above this pH the monoanionic form is predominant. Equation (4) can therefore be represented more completely in terms of these reactive species, as shown in the following series of equations;

$$H_2A \xleftrightarrow{k_{a1}} HA^- + H^+ \qquad (7)$$

$$HA^- \xleftrightarrow{k_{a2}} A^{2-} + H^+ \qquad (8)$$

$$[HCrO_4]^- \xleftrightarrow{k_c} [CrO_4]^{2-} + H^+ \qquad (9)$$

$$HA^- + [HCrO_4]^- \xrightarrow{k_1} \text{Products} \qquad (10)$$

$$HA^- + [CrO_4]^{2-} \xrightarrow{k_2} \text{Products} \qquad (11)$$

$$H_2A + [HCrO_4]^- \xrightarrow{k_3} \text{Products} \qquad (12)$$

$$H_2A + [CrO_4]^{2-} \xrightarrow{k_4} \text{Products} \qquad (13)$$

The overall rate law consistent with this is represented by

$$\text{Rate}=(k_1[HCrO_4^-] + k_2[CrO_4^{2-}])\,[HA^-] + (k_3[HCrO_4^-] + k_4[CrO_4^{2-}])\,[H_2A] \qquad (14)$$

After making the relevant substitutions for concentration of each species the following equation is obtained

$$k_{obs} = \frac{(k_1 k_{a1}[H^+] + k_2 k_{a1} k_c + k_3[H^+]^2)}{(k_{a1} + [H^+])(k_c + [H^+])}[A]_t + k_r \qquad (15)$$

Comparing equation (5) and (15) k_f is given by

$$k_f = \frac{(k_1 k_{a1}[H^+] + k_2 k_{a1} K_c + k_3[H^+]^2)}{(k_{a1} + [H^+])(k_c + [H^+])} \qquad (16)$$

Since k_r is a minor reaction it can be excluded from further analysis. It was found that the best fits for the rate constants were obtained by considering the k_1 and k_3 pathways to be most significant below pH 5.0 and k_1 and k_2 to be most significant above pH 5.0. According to Connett and Wetterhahn (1985), the pH dependence could be explained if the formation of the chromate-ascorbate ester intermediate is promoted by the presence of extra protons. The slower reactions at higher pH values could be due to OH- becoming the leaving group instead of water. But according to O'Brien and Woodbridge (1997), the small deviation from linearity of the plot of k_{obs} v's $[H^+]$ and the relatively large percentage error in k_1 and k_2 may suggest that the roll of $[H^+]$ is more complex than this.

Effect of Anaerobic Conditions.

Dixon and Sadler (1993), reported that under anaerobic conditions L-ascorbic acid rapidly reduced chromium (VI) at a rate which is about 10 times faster than the rates of reaction carried out in oxygen, and that the rate constants increased as oxygen concentration decreased. O'Brien and Woodbridge (1997), reported that the reaction showed no oxygen-dependence in demetallated HEPES or phosphate buffers at any pH (6.80-7.15). However, in Tris-HCl buffer the first-order-rate constants increased in a non linear fashion as oxygen concentration decreased and suggest that these results could be due to a buffer / trace metal ion effect rather than oxygen dependence.

This result shows that the reported oxygen-dependence of the chromate-ascorbic acid reaction by Dixon and Sadler (1993), was caused by the use of non-demetallated Tris-HCl buffer and was not an intrinsic feature of the redox reaction between chromium (VI) and ascorbic acid.

Formation of Cr (V) Intermediates.

Goodgame and Joy (1987), added milk to potassium dichromate ($K_2Cr_2O_7$) and using EPR spectroscopy observed the formation of Cr (V). The interaction of milk at room temperature gave, within a few minutes, a small, sharp, EPR band at g = 1.980, characteristic of Cr (V). The peak grew over a period of several hours and reached its maximum at approximately 1 day. The main g = 1.980 band was a multiplet, comprising four principle components with approximately 0.9 gauss spacing. It's set of four ^{53}Cr hyperfine bands were also observed and these had 17.9 gauss spacing, a value very similar to those observed for Cr(V) in oxygen donor atom environments. Vitamin C, ascorbic acid, which also reduces Cr(VI) to Cr (III) via Cr(V) species, is also present, but in much lower concentrations. Although the Cr(V) EPR band generated by ascorbic acid occurs at g = 1.979 it does not possess the multiplet structure observed for the Cr(V) complex in milk.

2.0. Experimental Procedures and Results.

2.1. Voltammetry.

2.1.1.(a). Introduction.

In this section the method of Wang (1997) for the determination of chromium(VI) using adsorptive catalytic stripping voltammetry has been modified and applied to the determination of chromium(VI) in a chromium enriched yeast extract. The supporting electrolyte used was a phosphate buffer at pH 6.0 containing cupferron. At an initial potential of –100mV (v Ag/AgCl) chromium(VI) is adsorbed on to the surface of a static mercury drop electrode as the chromium(III)-cupferron complex. On scanning in a negative direction a chromium reduction peak is observed at –1.0 V due to reduction of the metal centre in the complex..

The following experiments were carried out.

1. Polarogram of the Extracting Buffer.
2. Attempted Removal of the Peak from the Supporting Electrolyte by Solvent Extraction.
3. Attempted Removal of the Peak from the Supporting Electrolyte by Varying the Initial Potential.
4. Attempted Removal of the Peak from the Supporting Electrolyte by Varying Drop Growth Time.
5. Removal of Peak by EDTA.
6. The Effect of the Presence of Lead on the Supporting Electrolyte.
7. The Effect of Multiple Scanning on the Cr(VI) Peak Height.
8. The Effect of the Presence of Cr(III) on the Cr(VI) Peak.
9. The Effect of Passing Cr(III) Through an Anion Exchange Column.
10. The Effect of Electrolysis Time on Cr(VI) Peak Height.

11. Preparation of a Calibration Curve.
12. Application of Polarography to the Chromium Enriched Yeast Extract.
13. The Effect of the Extracting Buffer on the Supporting Electrolyte.

2.1.1.(b). Reagents and Solutions.

Reagents.

1. Sodium Dihydrogen Phosphate, $NaH_2PO_4.2H_2O$, F.W.=156.01 g mol^{-1}, May and Baker Ltd., #48933.
2. Cupferron, (N-nitroso-N-phenylhydroxylamine ammonium salt), $C_6H_5N(NO)ONH_4$ F.W.=155.16 g mol^{-1}, Aldrich Chemical Company Inc., #20688-1.
3. EDTA, Ethylenediamine Tetra-Acetic Acid, Disodium salt, F.W.=372.24 g mol^{-1}, BDH Chemicals Ltd., #28025.
4. Potassium Dichromate, $K_2Cr_2O_7$, F.W.=294.18 g mol^{-1}, Wardle Chemical Company Ltd., #P04868.
5. Chromium (III) Nitrate-9-Hydrate, $Cr(NO_3)_3.9H_2O$, F.W.=400.15 g mol^{-1}, Riedel-de Haen, #7097A.
6. Sodium Hydroxide, NaOH, F.W.=40.0 g mol^{-1}, Wardle Chemical Ltd., #S05652.
7. Zinc Sulphate, $ZnSO_4.7H_2O$, F.W.=287.54 g mol^{-1}, Wardle Chemical Ltd., #216718.
8. Amberlite IRA-410, Ionic Form Chloride, Strongly Basic Anion Exchanger, Sigma Chemical Company, Lot No. 87H0670.
9. Chromium Enriched Yeast, Alltech Ireland, Dunboyne, Co. Meath.
10. Hydrochloric Acid, HCl, F.W.=36.45g mol^{-1}, S.G.=1.18, BDH Laboratory Supplies.

11. Tris(hydroxymethyl)aminomethane, $C_4H_{10}NO_3$, F.W. = 121.14g mol^{-1}, Riedel-de-Haen, #33742.

12. Lead Nitrate, $Pb(NO_3)_2$, F.W.=331.21g mol^{-1}, Riedel-de-Haen, #71040.

Preparation of Solutions.

1. *Supporting Electrolyte - 2mM Phosphate, 1 x 10^{-4} M Cupferron.*

   2mM Sodium Dihydrogen Phosphate.

   A 0.0312g sample of sodium dihydrogen phosphate was dissolved in 100cm^3 of doubly distilled water.

   0.1 mol dm^{-3} Cupferron.

   A 0.3879g sample of cupferron was dissovled in 25cm^3 of doubly distilled water.

   A 100µL aliquot of this was added to 100cm^3 of the 2mM sodium dihydrogen phosphate. The pH of this solution was then adjusted to 6 using 0.1M NaOH.

2. *0.1M NaOH.*

   A 0.4001g sample of sodium hydroxide was dissolved in 100cm^3 of distilled water.

3. *0.01M EDTA.*

   A 0.3720g sample of ethylenediaminetetra-acetic acid, disodium salt, was dissolved in 100cm^3 of distilled water.

4. *Cr(VI) Standards.*

   (a). 1000ppm Cr(VI) in doubly distilled water.

   A 0.2829g sample of potassium dichromate was dissolved in 100cm^3 of doubly distilled water.

(b). 0.1ppm Cr(VI).

A 10cm^3 aliquot of 1000ppm Cr(VI) in doubly distilled water was diluted to 100cm^3 again with doubly distilled water.

5. *Cr(III) Standards.*

(a). 1000ppm Cr(III).

A 0.7696g sample of chromium (III) nitrate-9-hydrate was dissovled in 100cm^3 of doubly distilled water.

(b). 10ppm Cr(III).

A 1cm^3 aliquot of 1000ppm Cr(III) was diluted to 100cm^3 with doubly distilled water.

(c). 1.0ppm Cr(III).

A 10cm^3 aliquot of 10ppm Cr(III) was diluted to 100cm^3 with doubly distilled water.

(d). 0.1ppm Cr(III).

A 1cm^3 aliquot of 10ppm Cr(III) was diluted to 100cm^3 again with doubly distilled water.

6. *10ppm Zinc.*

(a). 1000ppm Zinc.

A 0.4398g sample of zinc sulphate was dissolved in 100cm^3 of doubly distilled water. A 1cm^3 aliquot of this was diluted to 100cm^3 with doubly distilled water.

7. *Anion Exchange Column.*

A 15g sample of amberlite IRA-410 was weighed and excess doubly distilled water was added. A well stirred slurry of the resin was then transferred into an exchange column insuring that no air bubbles were present.

8. 0.1M Hydrochloric Acid.

An 850μL aliquot of concentrated HCl was diluted to 100cm^3 with doubly distilled water.

9. 0.2M Tris NaOH pH 13.

A 12.1152g sample of tris(hydroxymethyl)aminomethane was dissolved in 500cm^3 of distilled water. The pH of this solution was adjusted to 13 using 5M NaOH.

10. 100ppm Pb.

(a). 1000ppm Pb.

A 0.1600g sample of lead nitrate were dissolved in 100cm^3 of doubly distilled water.

(b). 100ppm Pb.

A 10cm^3 aliquot of 1000ppm Pb was diluted to 100cm^3 with doubly distilled water.

2.1.1.(c). Instrumentation.

Radiometer Pol 150 Polarographic Analyzer.

Radiometer MDE 150 Polarographic Analyzer.

Deskjet 520 Hewlett Packard.

Nitrogen Cylinder BOC Ltd..

Fume Hood, Chemical Systems Control Ltd., Ashbourne, Co. Meath.

Dell Optiplex 466/Le.

Micropette, Eppendorf, 10μL.

Software, Trace Master 5 Window's 95.

Glassware.

All glassware was washed with 0.5% ultra pure nitric acid and rinsed with doubly distilled water prior to use. The polarographic cell was also rinsed with doubly distilled water prior to use and stored in KCl solution when not in use, as were the electrodes.

2.1.1.(d). Methods and Experiments.

The general procedure carried out in order to obtain a polarogram was as follows. $10cm^3$ of supporting electrolyte solution were transferred by pipette into a clean dry polarographic cell and purged for 4 minutes with nitrogen. The preconcentration potential (-100mV) was applied to a fresh mercury drop while the solution was stirred. Following the preconcentration period, the stirring was stopped and after 15 seconds potential was scanned in a negative direction using differential pulse polarography (scan rate of 20mV s^{-1} and pulse amplitude of 50mV). The scan was terminated at –1500mV. (Wang (1997)). Figure 2.1 represents a typical polarogram obtained for a 2 ppb solution of Cr(VI) during this work using the above procedure. The Cr(VI) peak was observed at approximately –1.0V.

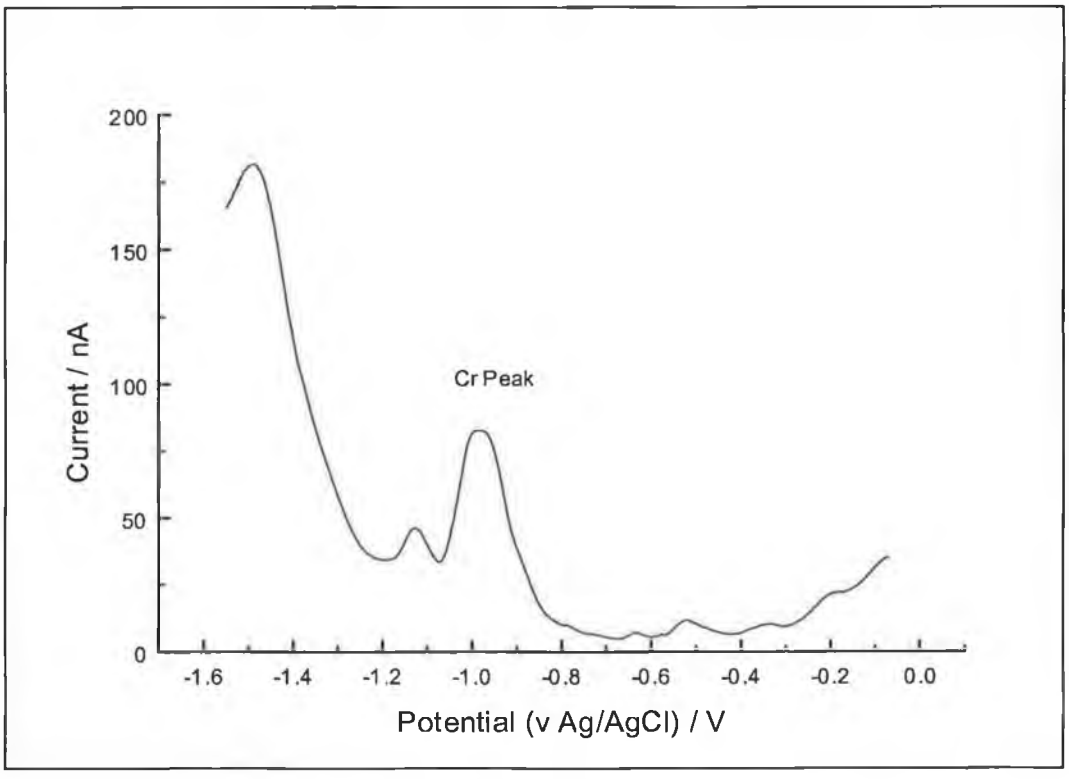


Figure 2.1. Typical polarogram obtained for Cr(VI) using the above procedure.

Software Parameters.

The following parameters were entered into the Trace Master 5 software prior to running the polarogram.

Operating Conditions - ASV/CSV.

Cell Parameters - Hanging Mercury Drop Electrode.

Number of drops = 3

Stirring rate (rpm) = 300

Purge Time = 240s.

Electrolysis = 30s.

Waiting Time = 15s.

Growth Time = 0.5s.

Signal Parameters -Differential Pulse Polarography.

Minimum current range = 10nA.

Maximum current range = 1mA.

E initial = -100mV.

E final = -1500mV.

Step duration = 0.1s.

Step amplitude = 2mV.

Pulse amplitude = 50mV.

These parameters were used throughout except for

(a). Effect of electrolysis time, when the electrolysis time was varied from 0s to 60s,

(b). Effect of E-initial, when E-initial was varied from -650mV to -950mV.

(c). Effect of Growth Time, when the growth time was varied from 0.1s to 2.5s.

1. Polarogram of the Supporting Electrolyte.

Figure 2.2 shows the polarogram obtained for the supporting electrolyte using the parameters set out above. Fig 2.2(a) shows the supporting electrolyte containing 2mM phosphate plus cupferron while Fig 2.2(b) shows the supporting electrolyte containing 2mM phosphate only. The peak at –1.0V in 2.2(b) is the expected location of the chromium peak since cupferron complexes with chromium to produce the chromium peak at approximately this potential. This peak was not reported in the polarogram of the supporting electrolyte in the original reference (Wang 1997) and so was thought to be due to contamination.

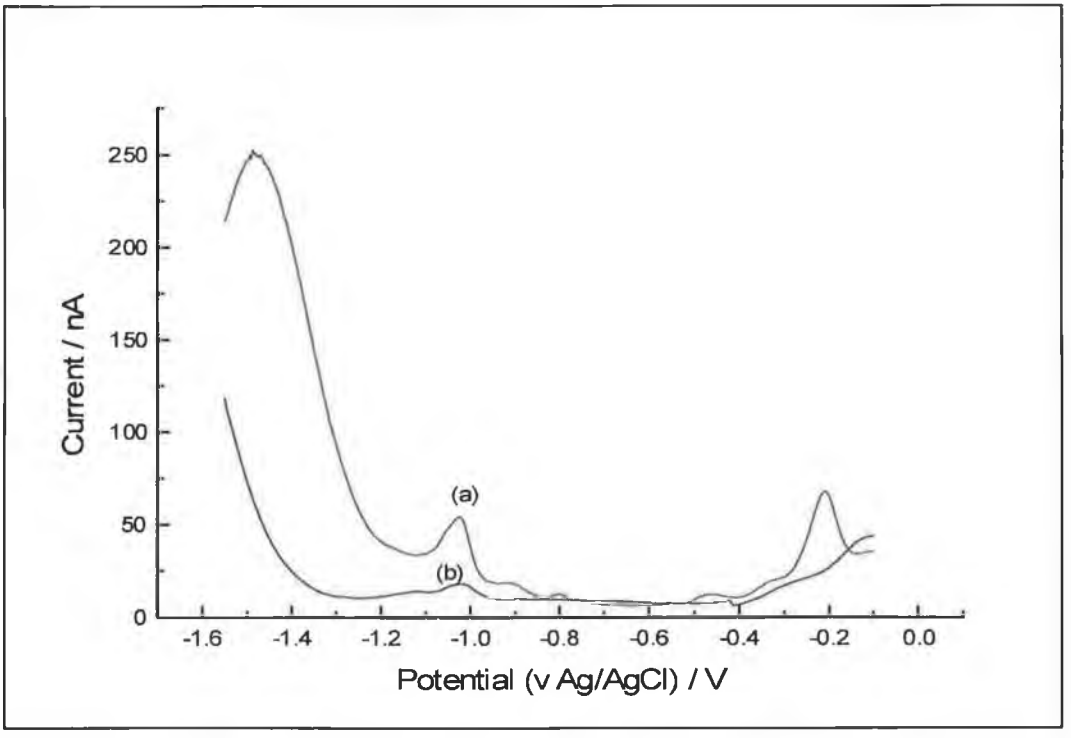


Figure 2.2. Polarograms of the supporting electrolyte. (a) 2mM Phosphate plus 1 x 10^{-4}M cupferron and (b) 2mM Phosphate.

2. Attempted Removal of Peak from Supporting Electrolyte by Solvent Extraction.

As it was suspected at this stage that the peak in the supporting electrolyte was due to a Cr(III)-cupferron complex which was likely to be uncharged, it was decided to try to remove the complex from the cupferron solution by solvent extraction. A $25cm^3$ aliquot of 0.1M cupferron was shaken with $25cm^3$ of chloroform. This solution was then heated on a hot plate until the excess chloroform was boiled off. The remainder of the solution was left to cool. Figure 2.3 shows the polarograms obtained for the supporting electrolyte containing cupferron (a) before solvent extraction and (b) after solvent extraction. From these polarograms it can be seen that the extraction of cupferron with chloroform did reduce the peak in the supporting electrolyte but it did not fully remove it.

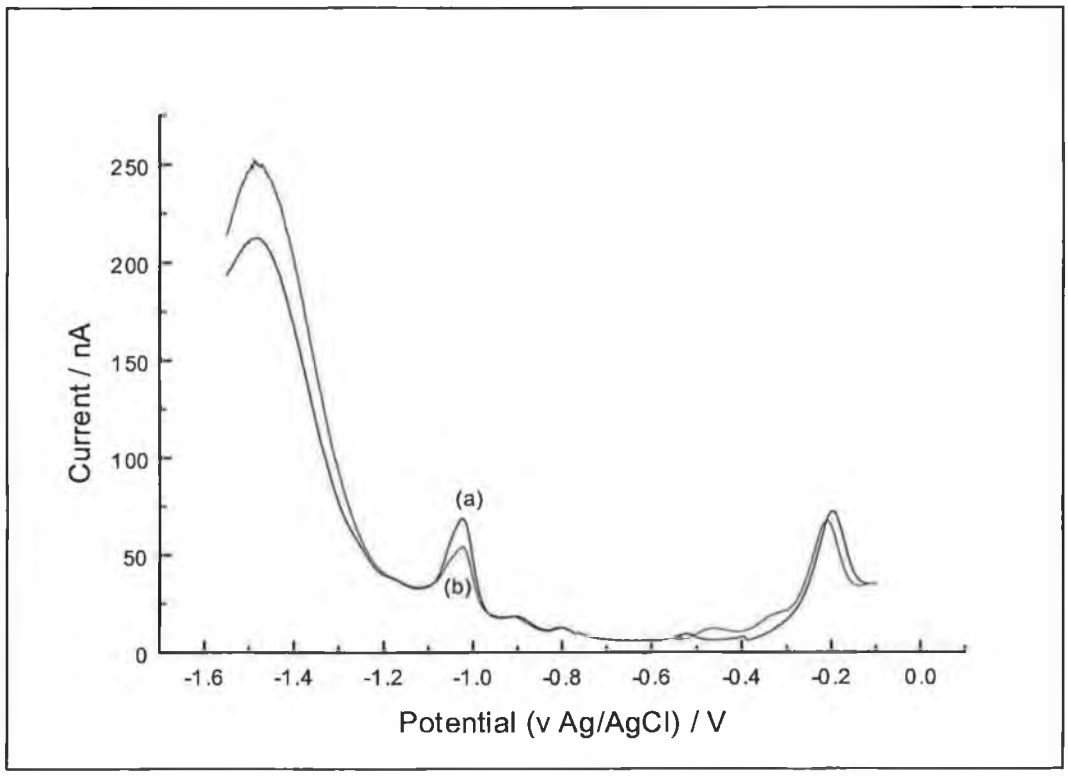


Figure 2.3. Polarograms of the Supporting Electrolyte containing Cupferron. (a) before extraction and (b) after extraction.

3. Attempted Removal of Peak from Supporting Electrolyte by Varying the Initial Potential.

$10cm^3$ of 2mM phosphate containing 1 x 10^{-4}M cupferron were scanned as above resulting in a peak at –1.0V. The initial potential (E_{init}) was then varied from –950mV to –650mV in 50mV increments and a polarogram was obtained after each increment. It was observed that as the initial potential became more negative the peak height decreased but was never removed fully. Data for this experiment is given in appendix 2.1.

4. Attempted Removal of Peak from Supporting Electrolyte by Varying Drop Growth Time.

$10cm^3$ of supporting electrolyte containing 1 x 10^{-4}M cupferron were scanned as above resulting in a peak appearing at –1.0V. The growth time was then varied from 0.1s to 2.5s and a polarogram was obtained after each variation. It was found that as the growth time increased so to did the peak at –1.0V in the supporting electrolyte. . Data for this experiment is given in appendix 2.2.

5. Removal of Peak using EDTA.

(a). The Effect of the Presence of Zinc on the Supporting Electrolyte.

At this stage it was suspected that the peak in the supporting electrolyte may have been due to zinc(II) contamination. $10cm^3$ of the supporting electrolyte (2mM phosphate, 1 x 10^{-4}M cupferron) were scanned as a blank using the parameters set out above. 100µL of 10ppm zinc were then added to the plarographic cell and scanned to produce a large peak at a potential of –1.0V (this peak would overlap with the Cr(VI)

peak). Figure 2.4 (a) shows the polarogram obtained when 100μL of 10ppm zinc were added to the supporting electrolyte.

(b). Addition of 100μM EDTA.

If the peak in the supporting electrolyte was due to zinc(II) then it should be possible to remove the peak using EDTA which forms a strong complex with zinc(II). 100μL of 0.01M EDTA were then added to the solution containing the added zinc and this solution was rescanned. This resulted in the reduction of the peak produced by the added zinc, indicating that the presence of 100μM of EDTA will remove zinc that may be present in the supporting electrolyte, and so produces a flat baseline at the potential at which the Cr(VI) peak appears. Figure 2.4 (b) shows the polarogram obtained in the presence of 100μM EDTA.

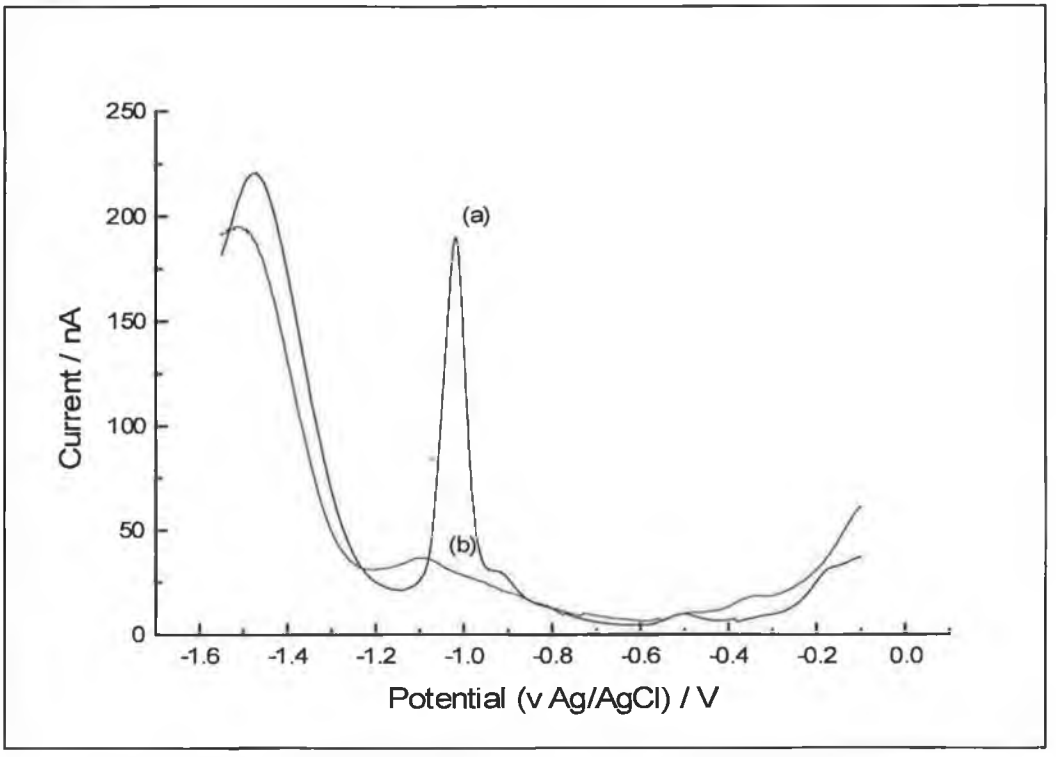


Figure 2.4. Polarograms of the Supporting Elecrolyte. (a) after addition of 100μL of 10ppm Zinc and (b) after addition of 100μM EDTA.

Consequently 2mM phosphate and 1 x 10^{-4}M cupferron containing 100μM EDTA as supporting electrolyte was used for all further analyses.

6. The Effect of the Presence of Lead on the Supporting Electrolyte.

$10cm^3$ of the supporting electrolyte containing 100μM EDTA were scanned as above. 100μL of 100ppm Pb were then added to the polarographic cell and scanned. No peak was obtained. Another 100μL of 100ppm Pb were added and scanned. Again no peak was observed, implying that the presence of lead had no effect on the supporting electrolyte.

7. The Effect of Multiple Scanning on Peak Height.

This experiment was performed to show that the quantity of chromium removed from the solution in the preconcentration step was a negligible fraction of the total chromium content. $10cm^3$ of the supporting electrolyte containing 100μM EDTA was scanned as a blank. An aliquot of Cr(VI) standard giving 3ppb Cr(VI) was added resulting in a peak at approximately -0.9mV. Five consecutive scans were then carried out on this solution. The average peak height was 99.4966 +/- 0.8828. This result indicates that Cr(VI) is not lost during multiple sacnning, and that continuous rescanning of a solution does not affect the Cr(VI) peak height. Data for this experiment is given in appendix 2.3.

8. The Effect of the Presence of Cr(III) on the Cr(VI) Peak.

It was reported in the original paper (Wang 1997) that Cr(III) may also be determined by this method. However in the procedure being developed here the supporting electrolyte contains $0.1x10^{-3}M$ EDTA. This should be capable of complexing the Cr(III) and may prevent Cr(III) from interfering with the determination of Cr(VI). $10cm^3$ of supporting electrolyte containing 100μM EDTA was scanned as a blank. 300μL of 0.1ppm Cr(III) were then added to the polarographic cell, to produce a 3ppb

Cr(III) solution in the cell. Figure 2.5 shows the polarogram obtained after the addition of 3ppb Cr(III) to the polarographic cell. A peak at approximately -0.9V indicates that the presence of Cr(III) will overlap with the Cr(VI) peak. The peak current from the 3ppb Cr(III) solution (84.3 nA) is approximately the same order of magnitude as the peak current (82.5 nA) from a 3ppb solution of Cr(VI). This indicates that EDTA is not effective in suppressing the response of the Cr(III). No experiments were carried out to determine if a larger excess of EDTA was more effective. This result indicates that the Cr(VI) and Cr(III) would first have to be separated before chromium(VI) could be analysed by this method.

9. *The Effect of Passing Cr(III) Through an Anion Exchange Column.*

In later experiments it was discovered that on standing in air Cr(III) can be oxidised to Cr(VI) (section 2.2.2.(c).). Since it would be necessary to separate Cr(III) and Cr(VI) prior to analysis it was proposed that Cr(VI) could be removed from a solution containing both Cr(III) and Cr(VI) by passing the mixture through an anion exchange column. Since Cr(III) has a positive charge it should pass through an anion exchange column, while Cr(VI) with a negative charge should adhere to it. To show that Cr(III) passes through the column the following experiment was carried out. A $10cm^3$ aliquot of the 1ppm Cr(III) was passed through an anion exchange column, as prepared in section 2.1.1.(b)., collected along with $50cm^3$ of rinsings (doubly distilled water) and made up to $100cm^3$ again with doubly distilled water giving a 0.1 ppm solution of Cr(III). A $10cm^3$ aliquot of the supporting electrolyte containing 100μM EDTA was scanned as a blank using the parameters set out above. 300μL of the 0.1ppm Cr(III) was added to the cell and the polarogram recorded. Figure 2.5 shows the polarograms obtained for the analysis of Cr(III) not passed through the anion exchange column (a)

and passed through the anion exchange column (b), together with the polarogram of blank solution (c).

A slight peak at –0.9V was observed for the situation where Cr(III) was passed through the column indicating that most of the chromium had been lost in the ion exchange process. Ion exchange separation of Cr(VI) and Cr(III) was studied later and is described in section 2.3.9 (p 175).

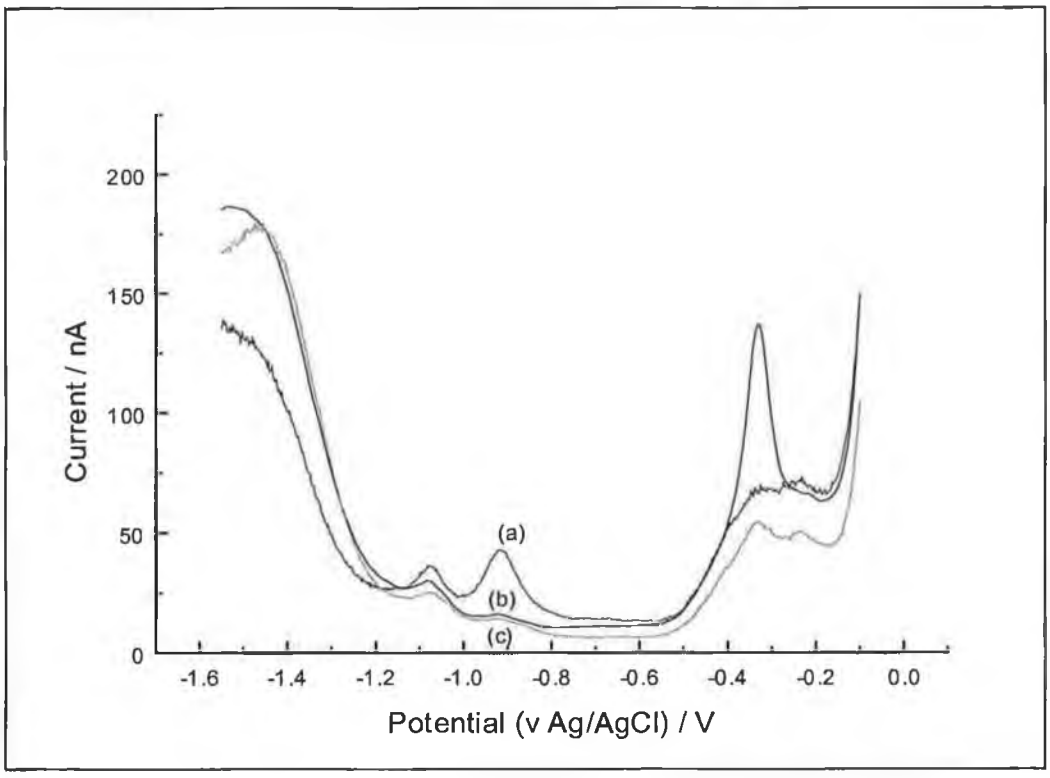


Figure 2.5. Polarograms of 3ppb Cr(III) (a) before being passed through an anion exchange column and (b) after being passed through an anion exchange column. (c) is the supporting electrolyte.

10. The Effect of Electrolysis Time on the Cr(VI) Peak Height.

This experiment was carried out to see if the Cr(VI) peak height increased or decreased as the electrolysis time was varied. $10cm^3$ of supporting electrolyte containing 100μM EDTA were scanned as a blank. An aliquot of Cr(VI) was then

added to the polarographic cell to produce a 5ppb Cr(VI) concentration and the solution was scanned with the electrolysis time varying from 0 to 60seconds in 10 second increments. A polarogram was obtained after each increment. This resulted in the Cr(VI) peak height increasing as the electrolysis time increased up to 30 seconds, then the Cr(VI) peak did not increase further. An optimum electrolysis time of 30 seconds was selected. Data for this experiment is given in appendix 2.4.

11. Preparation of a Calibration Curve.

A calibration curve was prepared from 0 to 5ppb Cr(VI) by addition of 100µl aliquots of 0.1ppm chromium(VI) to 10 cm^3 of the supporting electrolyte containing 100µM EDTA. This resulted in an increase in the peak current as the volume of Cr(VI) added to the cell increased. Figure 2.6 shows the polarograms obtained after the addition of each aliquot of Cr(VI).

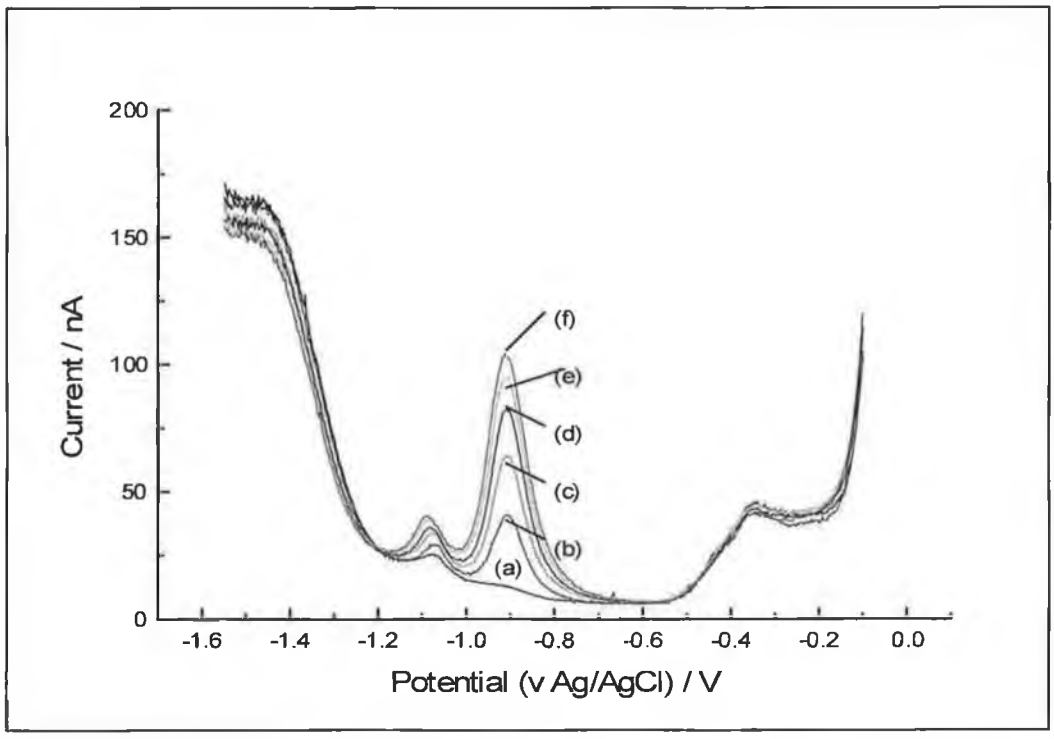


Figure 2.6. Polarograms of Cr(VI) Standards. (a) Supporting Electrolyte, (b) 1ppb, (c) 2ppb, (d) 3ppb, (e) 4ppb and (f) 5ppb Cr(VI).

A calibration curve from 0 to 5ppb Cr(VI) was produced. Data from this experiment is given in appendix 2.5.

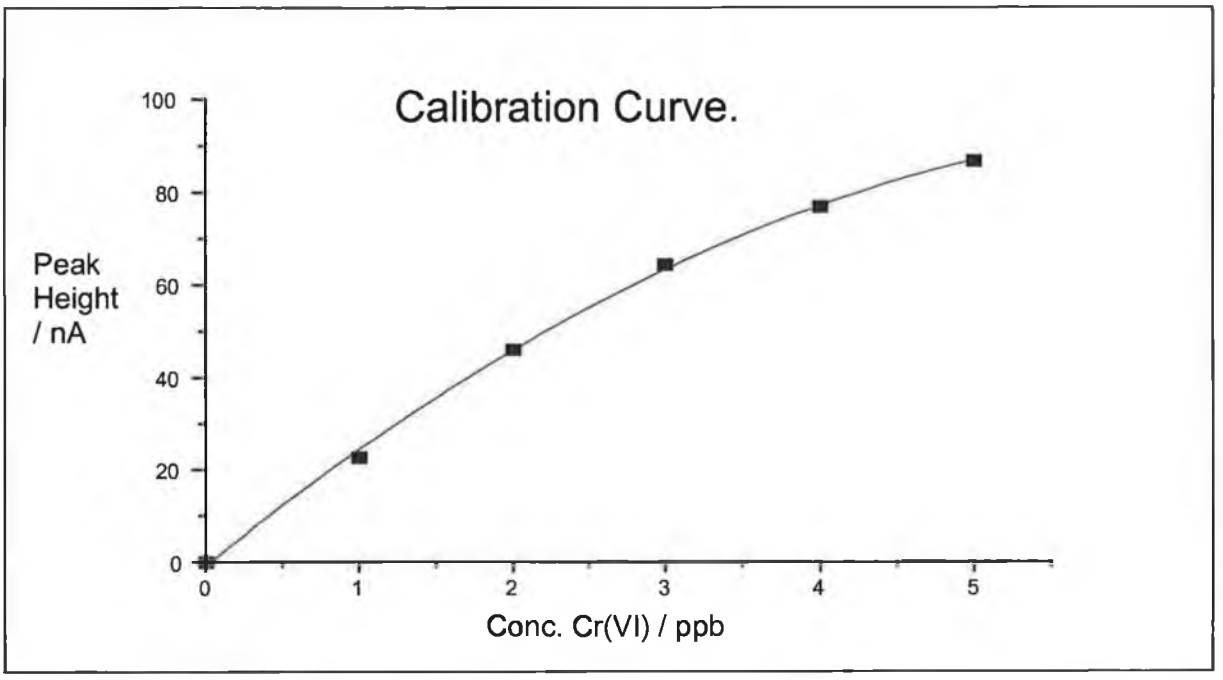


Figure 2.7. Calibration Curve for Polarography.

The calibration curve was non-linear and the data was fitted to a second order polynomial.

Table 2.1. Data from calibration curve for polarography.

Parameter	Value
A_0	0.32142857±1.03242
A_1	29.517857±0.97113
A_2	-2.3035714±0.18643
R	0.99967
R^2	0.99934
SD	1.13913

12. Application of Polarography to Chromium Enriched Yeast Extract.

The extraction procedure described in Section 2.2.1.(c) was carried out on 1.0010g of chromium enriched yeast. A $1cm^3$ aliquot of the extract was then diluted to $100cm^3$ with doubly distilled water and the pH was adjusted to 3.8 (same as that of the Cr(III)

in experiment 9 above) using 0.1M HCl. 10cm^{3}of supporting electrolyte containing 100μM EDTA were scanned as a blank using the parameters set out above. 100μL of the 1 in 100 dilution of the yeast extract was then added to the polarographic cell and scanned, resulting in a peak at –0.9V. This peak could be due to Cr(VI) or it could be Cr(III) since the extraction procedure extracts Cr(III) and Cr(VI). In order to remove Cr(VI) from the yeast extract 100cm^{3}of the extract was passed through an anion exchange column as described above. 100μL of the eluent was then analysed as above again resulting in a peak at –0.9V. The peak was reduced compared to that of the yeast extract not passed through the anion exchange column. Figure 2.8 shows the polarograms obtained for analysis of the yeast extract (a) supporting electrolyte (b) passed through an anion exchange column and (c) not passed through an anion exchange column.

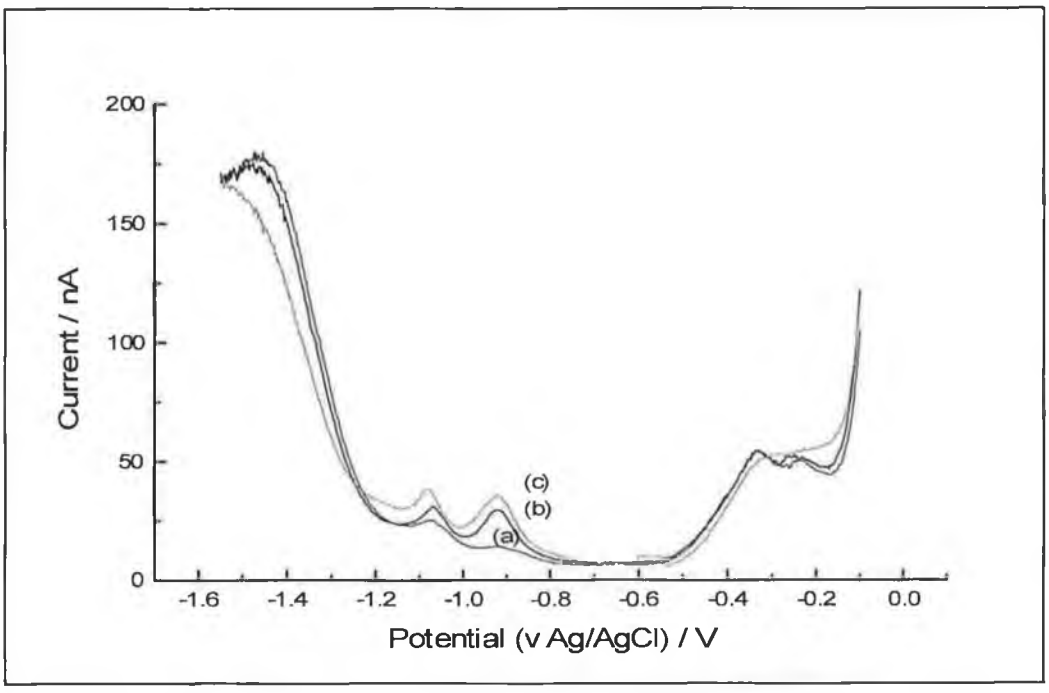


Figure 2.8. Polarograms of enriched yeast (a) supporting electrolyte (b) after being passed through an anion exchange column (c) before being passed through an anion exchange column.

The experiment does not prove that there was Cr(VI) in the yeast extract. The reduction in the peak height may not be due to removal of Cr(VI) but also to loss of Cr(III) as discussed in experiment 9 above. At the time of these experiments it was not appreciated that Cr(III) was lost in the ion exchange process. The fact that Cr(III) and Cr(VI) are both polarographically active requires a more reliable separation of the Cr(VI) prior to analysis. The ion-exchange process requires further study.

13. The Effect of the Extracting Buffer on the Supporting Electrolyte.

$10cm^3$ of the supporting electrolyte containing 100μM EDTA were scanned as a blank using the parameters set out above. 100μL of 2×10^{-3}M Tris-NaOH pH 13 were added and scanned. No peak appeared after the addition of the Tris-NaOH implying that the extracting buffer does not contain Cr(VI) and so is not responsible for the peak at –0.9V in experiment 11 above.

2.1.2. Graphite Furnace Atomic Absorption Spectroscopy.

2.1.2.(a). Introduction.

In this section the method from the Analytical Methods for Graphite Tube Atomizers (Varian 1982) has been modified by adding in an extra step at the end of the ash stage to completely remove matrix components prior to the atomisation stage. The final temperature used in the atomisation stage was lower than that recommended since it was found that the recommended temperature caused the graphite tube to burn out.

2.1.2.(b). Reagents and Solutions.

Reagents.

1. Ultra Pure Nitric Acid, HNO_3, F.W.= 63.01 g mol^{-1}, Aldrich Chemical Company Inc., #25812-1.
2. Potassium Dichromate, $K_2Cr_2O_7$, F.W.=294.18 g mol^{-1}, Wardle Chemicals Ltd., #P04868.

Preparation of Solutions.

1. *0.5% Nitric Acid.*

   5cm^3 of ultra pure nitric acid was diluted to 1 dm^3 with distilled water.

2. *Cr(VI) Standards.*

   (a). 1000ppm Cr(VI).

   0.2829g of $K_2Cr_2O_7$ was dissolved in 100cm^3 of 0.5% nitric acid.

   (b). 50ppm Cr(VI) .

   5cm^3 of 1000ppm Cr(VI) was diluted to 100cm^3 with 0.5% nitric acid.

   (c). 0.5ppm Cr(VI).

   1cm^3 of 50ppm Cr(VI) was diluted to 100cm^3 with 0.5% nitric acid.

(d). 0.05ppm Cr(VI).

$10cm^3$ of 0.5ppm Cr(VI) was diluted to $100cm^3$ with 0.5% nitric acid.

2.1.2.(c). Instrumentation.

Spectra AA-10 Atomic Absorption Spectrometer.

Varian GTA-96 Graphite Tube Atomiser.

Chromium Hollow Cathode Lamp, Pye Unicam Ltd., #39804.

Seikosha SL-SOIP Printer.

Sample Holders, JVA Analytical Ltd..

Glassware.

All glassware was washed with 0.5% ultra pure nitric acid and rinsed with distilled water prior to use. The sample holders were immersed in 0.5% ultra pure nitric acid for 24 hours prior to use.

2.1.2.(d). Procedures and Results.

1. Preparation of a Calibration Curve.

Using a blank of 0.5% nitric acid (a sample holder was filled with 0.5% nitric acid and placed in the slot named blank), a standard of 50μg dm^{-3} Cr(VI) in 0.5% nitric acid (another sample holder was filled with the 50μg dm^{-3} Cr(VI) and placed in the slot named standard), and using the following parameters a calibration curve was prepared from 0 to 50ppb Cr(VI). The required aliquots for the standard page are given in Table 2.2 and the furnace parameters are given in Table 2.3. The modifier slot was left empty.

Table 2.2. Chromium Standards for G.F.A.A. Spectroscopy.

	Volume of Blank/(μL)	**Volume of 0.05ppm Cr(VI) Standard (μL)**
Blank	20	0
Addition 1	16	4
Addition 2	12	8
Addition 3	8	12
Addition 4	4	16
Addition 5	0	20

Furnace Parameters are given in Table 2.2.

Table 2.3. Furnace Parameters for G.F.A.A. Determination of Chromium.

Step No.	**Temperature (^{o}C)**	**Time (seconds)**	**Gas Flow (dm^{-3} min^{-1})**	**Gas Type**	**Read**
1	75	5	3	Normal	No
2	90	60	3	Normal	No
3	120	10	3	Normal	No
4	120	2	0	Normal	No
5	1200	1	0	Normal	No
6	1200	15	0	Normal	No
7	2500	1.2	0	Normal	Yes
8	2500	2	0	Normal	Yes
9	2500	1.2	3	Normal	No

Result.

Using the above parameters a linear calibration curve was produced from 0 to 50ppb Cr(VI). The data for the calibration curve is given in appendix 2.6.

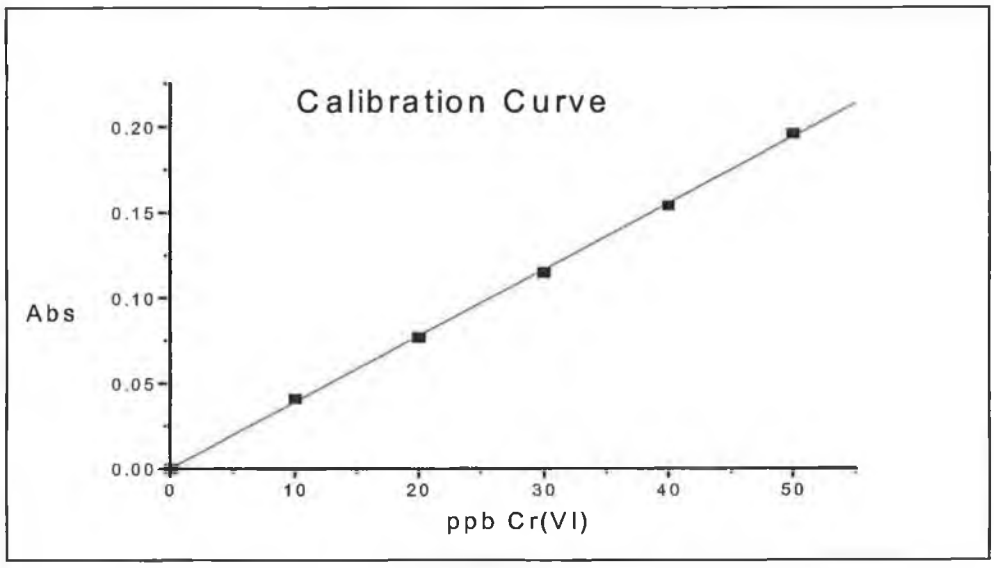


Figure 2.9. Calibration Curve for Graphite Furnace Atomic Absorption Spectroscopy.

Table 2.4. Data for calibration curve for G.F.A.A.S.

Parameter	Value
A	0.00024±0.00128
B	0.00388±0.00004
R	0.99976
SD	0.00176
N	6
P	8.3511×10^{-8}

2. Analysis of the Chromium Enriched Yeast Extract.

1.0008g of the enriched yeast was extracted as in section 2.2.1.(c) and a 1×10^{-5} dilution of this extract was made. A sample holder was then filled with this diluted extracted solution and using the parameters set out in Table 2.3 this solution was analysed. This resulted in an absorbance of 0.1089, implying that the diluted enriched yeast extract contained 28μg l^{-1}. The concentration of the chromium in the extract was calculated as 2.8 gl^{-1} = 2800 mgl^{-1} = 2800 ppm. This is total extracted chromium.

2.1.3. U.V. Spectroscopy.

2.1.3.(a). Introduction.

The procedure on which this section is based is a standard method for the detection of Cr(VI) in water analysis (published by the American Public Health Association, American Water Works Association, and Water Pollution Control Association (1971)). The reaction between chromate and diphenylcarbazide is a widely known and used one for the determination of chromium. This reaction is specific for Cr(VI) and so is a suitable method for the detection of Cr(VI) in the enriched yeast sample under investigation here since, the excess Cr(III) in the yeast extract will not interfere with Cr(VI) detection. An excess of Cr(III) was also used in the preparation of the standards for the calibration curve to simulate that of the enriched yeast. In order for this reaction to take place the reaction medium must be sufficiently acidic (approximately pH 2) for the colorimetric reaction to occur. Therefore H_2SO_4 was added to the diphenylcarbazide solution prior to the reaction with Cr(VI). It is also known that the stability of the colour decreases with time. In this section this decrease was studied. The Cr(VI)-DPC complex absorbs at 540nm.

Experiments carried out in this section include;

1. Preparation of a Calibration Curve.
2. The Effect of Time on the Absorbance of the Cr(VI)-DPC Complex.
   (a). Over Hours.
   (b). Over Days.

2.1.3.(b). Reagents and Solutions.

Reagents.

1. 1,5-Diphenylcarbazide, $C_{13}H_{14}N_4O$, F.W.=242.3g mol^{-1}, Aldrich Chemical Company Inc., #25922-5.

2. Potassium Dichromate, $K_2Cr_2O_7$, F.W.=294.19g mol^{-1}, Wardle Chemical Company Ltd., #PO4868.

3. Tris(hydroxymethyl)-aminomethane, $C_4H_{10}NO_3$, F.W.=121.14g mol^{-1}, Riedel-de Haen, #33742.

4. Chromium(III) Chloride, $CrCl_3.6H_2O$, F.W.=266.45g mol^{-1}, Riedel-de Haen, #12228.

5. Sodium Hydroxide, NaOH, 40.00g mol^{-1}, Wardle Chemical Company Ltd., #SO5652.

Preparation of Solutions.

1. *1,5-Diphenylcarbazide Solution. (1.67 x 10^{-3}M DPC, 1.47M H_2SO_4)*

   40cm^3 of concentrated H_2SO_4 were added to 360$cm^3$of doubly distilled water. 0.2027g of 1,5-diphenylcarbazide were dissolved in 100$cm^3$of ethanol. These two solutions were mixed with constant stirring. This solution was stored in the dark as light caused discolouration. When discolouration occurred fresh solutions were prepared, i.e., approximately every two weeks.

2. *5M NaOH.*

   20.0g of sodium hydroxide were dissolved in 100$cm^3$of distilled water.

3. *0.2M Tris-NaOH pH 13.*

   As in section 2.1.1.(b).

4. *40ppm Cr(III) in Tris-NaOH.*

   0.0512g of chromium(III) chloride were dissolved in 250cm^{3}of 0.2M Tris-NaOH pH13.

5. *Cr(VI) Standards.*

   (a). 1000ppm Cr(VI).

   0.2829g of potassium dichromate were dissolved in 100cm^{3}of distilled water.

   (b). 100ppm Cr(VI).

   10cm^{3}of 1000ppm Cr(VI) were diluted to 100cm^{3}with distilled water.

   (c). 10ppm Cr(VI).

   10cm^{3}of 100ppm Cr(VI) were diluted to 100cm^3 with distilled water.

2.1.3.(c). Instrumentation.

UV-160, UV-Visible Recording Spectrophotometric, Shimadzu.

Glassware.

All glassware was washed with 0.5% ultra pure nitric acid and rinsed with doubly distilled water prior to use.

2.1.3.(d). Procedures and Results.

The following parameters were set up in order to obtain the spectrum and the absorbance of the Cr-DPC complex at 540nm in this section.

(a). To obtain the spectrum of a solution.

Operating Parameters.

Spectrum

1. Meas. Mode Abs
2. Scanning Range 800-400nm.
3. Rec Range 0.00A-2.00A
4. Scan Speed Fast
5. No. of Scans 1
6. Display Mode Sequential

Following this the reference cell and the sample cell were filled with distilled water and these cell were placed in the required slots. Then the instrument was zeroed by pressing AUTO ZERO.

Next the sample cell was filled with the solution to be analysed (i.e. the Cr-DPC complex plus excess Cr(III)) and START/STOP was pressed. After the spectrum was recorded and printed, RETURN followed by MODE was pressed.

(b). To obtain the absorbance measurement of a solution.

After the spectrum of the solution had been produced, PEAK was pressed and the absorbance measurement at 540nm appeared on the screen. This measurement was then printed by pressing PRINT.

These parameters were used to produce a spectrum and the absorbance measurement of the Cr-DPC complex

1. Preparation of Calibration Curve.

A calibration curve from 0 to 1ppm Cr(VI) in 0.1ppm increments was obtained, using the parameters set out above by preparing the standards listed in Table 2.5. The concentration of the Cr(III) was chosen so as to simulate the presence of excess Cr(III) in the yeast extract. The DPC reagent was added to the Cr(VI) followed by the addition of the Cr(III) and dilution to $50cm^3$ with doubly distilled water. Each of these solutions was allowed to stand for 5 minutes to allow the complete formation of the Cr-DPC complex (only Cr(VI) reacts) prior to obtaining the spectrum or absorbance measurement at 540nm.

Table 2.5. Cr(VI) Standards.

10ppm Cr(VI) / cm^3	**1.6 x 10^{-3}M DPC / cm^3**	**Volume of 40ppm Cr(III) in Tris NaOH / cm^3**	**Total Volume / cm^3**
0.5	5	10	50
1	5	10	50
1.5	5	10	50
2	5	10	50
2.5	5	10	50
3	5	10	50
3.5	5	10	50
4	5	10	50
4.5	5	10	50
5	5	10	50

It was found that as the Cr(VI) concentration increased so to did the absorbance measurements and a linear calibration curve from 0 to 1ppm Cr(VI) was obtained (figure 2.10). Data for this experiment are given in appendix 2.7.

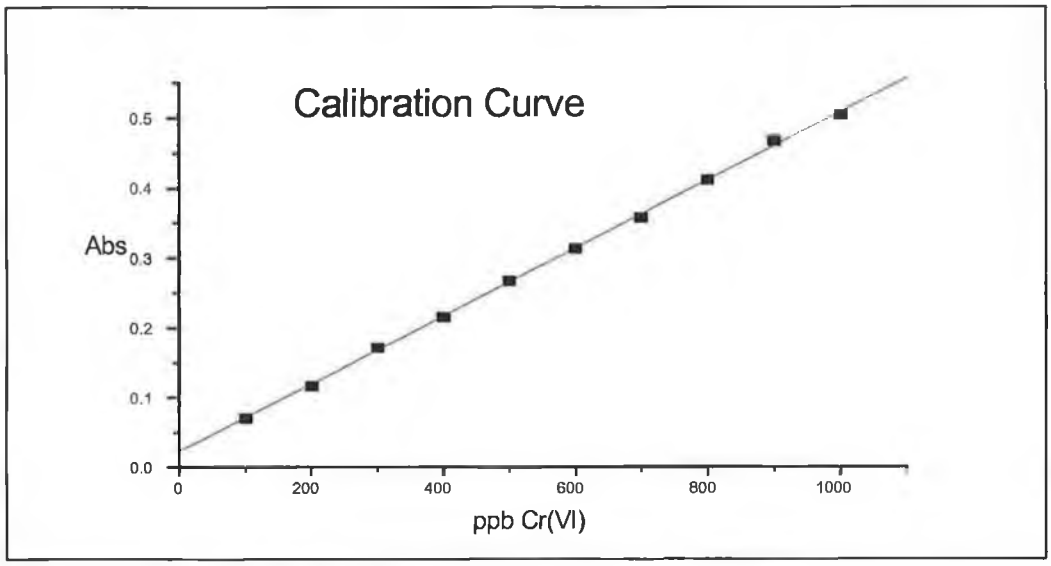


Figure 2.10. Calibration Curve for U.V. Spectroscopy.

Table 2.6. Data from calibration curve for U.V. spectroscopy.

Parameter	Value
A	0.01541±0.000407
B	0.0005±6.8861 x 10^{-6}
R	0.99913
SD	0.00722
N	11
P	6.6402 x 10^{-14}

2. The Effect of Time on Absorbance of the Cr-DPC complex.

(a). Over Hours.

A 500 ppb Cr(VI) standard solution was prepared by pipetting 2.5cm^3 of 10ppm Cr(VI), 2.5cm^3 of DPC and 10cm^3 of Cr(III) in Tris-NaOH into a 50cm^3 volumetric flask and making up to the mark with doubly distilled water. After 5 minutes, and every hour following, the spectrum of this solution was analysed and the absorbance was measured again at 540nm using the parameters set out above. It was found that

the absorbance measurements decreased linearly over time. Figure 2.11 represents the absorbance measurements obtained for this experiment and the data is given in appendix 2.8.

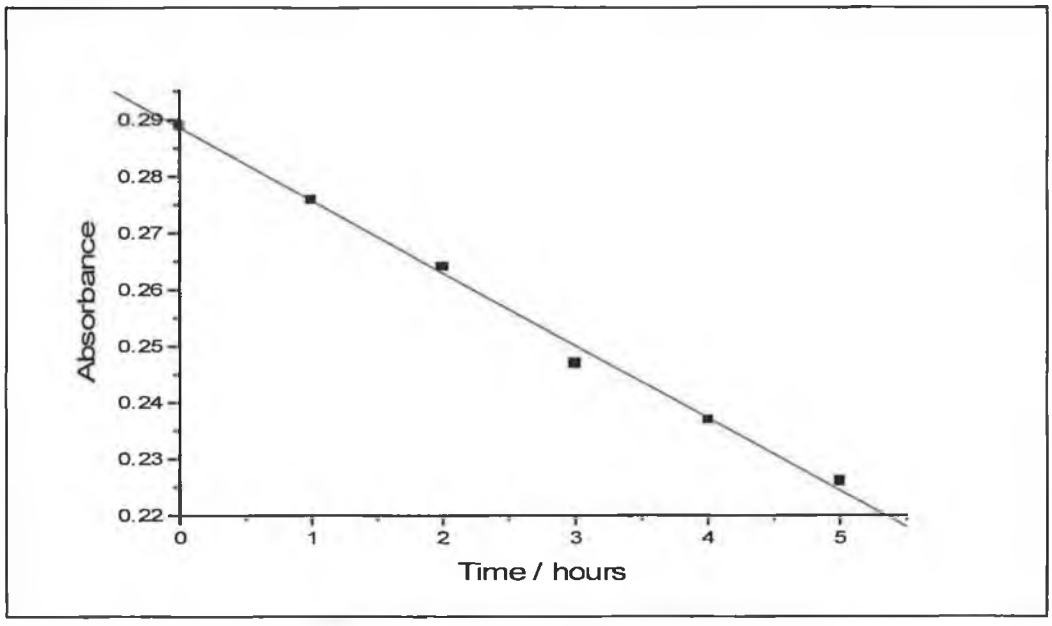


Figure 2.11. Effect of time on absorbance of Cr-DPC complex (hours).

Table 2.7. Data from graph showing the effect of time on absorbance of Cr-DPC complex (hours).

Parameters	Values
A	0.28857±0.00133
B	-0.01283±0.00044
R	-0.99767
SD	0.00184
N	6
P	8.1585×10^{-6}

From this graph the percentage decrease in absorbance measurement over 1 hour was calculated as 4.45%. It has been recommended that the absorbance of solutions containing DPC should be taken no longer than 15 minutes after the addition of the DPC to the solution as a decrease in absorbance measurement is obtained after this time limit.

(b). Over Days.

This solution was then analysed every 24 hours for 6 days. It was found that the absorbance of the Cr-DPC complex decreased expontenially over the 6 days and reached zero absorbance after the 6 days. This result is represented in figure 2.12 and the data is given is appendix 2.9.

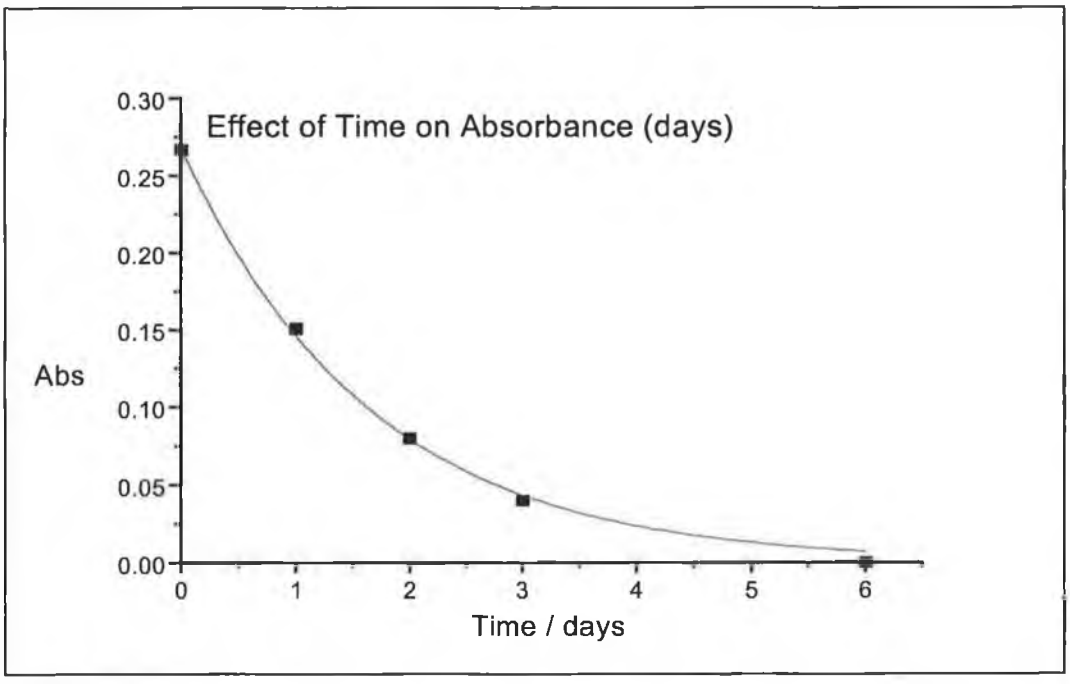


Figure 2.12. Effect of time on absorbance of Cr-DPC complex (days).

Table 2.8. Data from graph showing the effect of time on absorbance of Cr-DPC complex (hours).

Parameters	Values
Chi	2.8475×10^{-5}
Tolerance	0.05
A	0.26889±0.00515
B	0.60815±0.024

The graph above was fitted to the function A*EXP(-B*X) and the reaction was found to be first order with a rate constant of 0.60815 $days^{-1}$.

2.1.4. High Performance Liquid Chromatography.

2.1.4.(a). Introduction.

In this section a modification of the method of Padarauskas (1998), which used on-line preconcentration and determination of Cr(VI) in water by high-performance liquid chromatography with pre-column complexation with 1,5-diphenylcarbazide, was used for the determination of Cr(VI) in the chromium enriched yeast extract. Padauraskas et al. used a dual column technique where the first column was used for preconcentration and the second column was used for the chromatography. In the modification described here the procedure is carried out on a single column. Chromate was preconcentrated on a C_{18} column by passing the extract containing the Cr(VI) through the column for a predetermined length of time after complexation with diphenylcarbazide. After this preconcentration step the sample was eluted from the column using a solution of 6 x 10^{-3} mol dm^{-3} H_2SO_4 and 20% acetonitrile. Direct spectrophotometric detection at 546nm was used to detect the eluted Cr-DPC complex. It was found that the Cr-DPC peak generally eluted between 11 and 12 minutes. Figure 2.13. represents a typical chromatogram obtained using the above preconcentration and elution prodecure for standard 15ppb Cr(VI).

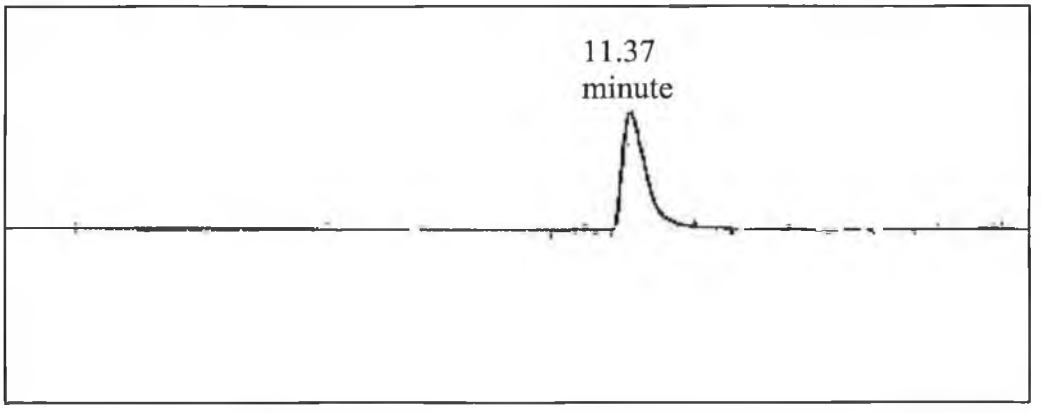


Figure 2.13. Typical chromatogram obtained for 15ppb Cr(VI).

2.1.4.(b). Reagents and Solutions.

Reagents.

1. Sulphuric Acid, H_2SO_4, S.G.=1.84, F.W.=98.07g mol^{-1}. Romil Ltd., Code A-9691.
2. Acetonitrile, CH_3CN, F.W.= 41.05g mol^{-1}, BDH Chemicals Ltd., #29220.
3. 1,5-Diphenylcarbazide, $C_{13}H_{14}N_4O$, F.W.=242.3g mol^{-1}, Aldrich Chemical Company Inc., #25922-5.
4. Potassium Dichromate, $K_2Cr_2O_7$, F.W.=294.19g mol^{-1}, Wardle Chemical Company, Ltd., #PO4868.
5. Chromium (III) Chloride, $CrCl_3.6H_2O$, F.W.=266.45g mol^{-1}, Riedel-de Haen, #12228
6. Tris(hydroxymethyl)-aminomethane, $C_4H_{10}NO_3$, F.W.=121.14g mol^{-1}, Riedel-de Haen, #33742.

Preparation of Solutions.

1. *Mobile Phase: 6 x 10^{-3}M H_2SO_4 in 20% Acetonitrile.*

   A 327µL aliquot of concentrated H_2SO_4 was added to 300cm^3 of distilled water, 200µL of acetonitrile was added and this solution was diluted to 1 dm^3 with distilled water,

2. *2 x 10^{-3}M DPC in 10% Acetonitrile, 0.048M H_2SO_4.*

   A 10cm^3 aliquot of acetonitrile was diluted to 100cm^3 with distilled water. 0.0485g of 1,5-diphenylcarbazide were dissolved in this and 261.0µL of concentrated H_2SO_4 were added.

3. *Cr(VI) Standards.*

   (a). 10ppm Cr(VI).

   As in section 2.1.3.(b).

(b). 100ppb Cr(VI).

1cm^3 of 10ppm Cr(VI) was diluted to 100cm^3 with distilled water.

(c). 5, 10, 15, 20 and 25 ppb Cr(VI).

5, 10, 15, 20 and 25 cm^3 of 100ppb Cr(VI) were diluted to 100cm^3 with distilled water.

4. Cr(III*) Standards in 0.2M Tris-NaOH pH 13.*

(a). 1000ppm Cr(III) in 0.2M Tris-NaOH pH 13.

0.5128g of chromium (III) chloride was dissolved in 100cm^3 of 0.2M Tris-NaOH pH 13.

(b). 500ppb Cr(III) in 0.2M Tris-NaOH pH 13.

50µl of 1000ppm Cr(III) was diluted to 100cm^3 with 0.2M Tris-NaOH pH 13.

(c). 15ppb Cr(III) in 0.2M Tris-NaOH pH 13.

3cm^3 of 500ppb Cr(III) was diluted to 100cm^3 with 0.2M Tris-NaOH pH 13.

7. Cr(VI) Standards in Excess Cr(III).

(i). 15ppb Cr(VI) and 15ppb Cr(III) in 0.2M Tris-NaOH pH 13.

15cm^3 of 100ppb Cr(VI) was pipetted into a 100cm^3 volumetric flask, some distilled water was added along with 15cm^3 of 100ppb Cr(III) and made up to the mark with 0.2M Tris-NaOH pH 13.

(ii). 15ppb Cr(VI) and 1.5ppm Cr(III) in 0.2M Tris-NaOH pH 13.

15cm^3 of 100ppb Cr(VI) was pipetted into a 100cm^3 volumetric flask, some distilled water was added along with 150µl of 1000ppm Cr(III) and made up to the mark with 0.2M Tris-NaOH pH 13.

(iii). 15ppb Cr(VI) and 50ppm Cr(III) in 0.2M Tris-NaOH pH 13.

15cm^3 of 100ppb Cr(VI) was pipetted into a 100cm^3 volumetric flask, some distilled water was added along with 5cm^3 of 1000ppm Cr(III) and made up to the mark with 0.2M Tris-NaOH pH 13.

2.1.4.(c). Instrumentation.

UV Spectrophotometric detector SPD-6A, Shimadzu.

Liquid Chromatography LC-6A, Shimadzu.

3390A Integrator – Hewlett Packard Model 1.

Alltech C_{18} Column I.D. = 4.6mm, length 250mm.

Glassware.

All glassware was washed with 0.5% ultra pure nitric acid and rinsed with distilled water prior to use.

2.1.4.(d). Procedures and Results.

The general procedure carried out in order to obtain a chromatogram in this section was as follows. A 2.5 cm^3 aliquot of 2 x 10^{-3}M DPC in 10% acetonitrile, 0.048M H_2SO_4 was added to 20cm^3 of the solution to be analysed. After 6 minutes (the time taken for the Cr-DPC complex to form) the solution was preconcentrated on the C_{18} column by passing the complexed sample solution through the column for 8 minutes at a flow rate of 1.5cm^3 min^{-1}. Following this preconcentration step analysis of the solution was carried out using 6 x 10^{-3}M H_2SO_4 in 20% acetonitrile as the mobile phase and the Cr-DPC complex was eluted from the column over a 30 minute period. The appearance of the Cr-DPC complex was signalled using on-line photometric detection at 546 nm. Attenuation was set at 0.16 and absorbance range was 0.64

(except when the Cr-DPC complex peak was offscale and then the absorbance range was 1.28).

1. The Effect of Absorbance Range Setting on Cr-DPC Peak.

In order to find the optimum absorbance range to use, the above procedure was carried out using 1 ppm Cr(VI) standard complexed with DPC as described above. The following variations were carried out on the absorbance setting: 1.28, 0.64, 0.32 and 0.16 and the peak area for the Cr-DPC complex eluted each time was recorded. It was found that as the absorbance range decreased the area of the Cr-DPC complex peak increased. The peak areas obtained here are given in Table 2.9.

Table 2.9. Peak Areas of Cr-DPC Complex obtained after Variation of the Absorbance Setting.

Absorbance Setting	Cr-DPC Complex Peak Area
1.28	21414
0.64	43501
0.32	89958
0.16	181140

An absorbance setting of 0.64 was generally used during analysis in this section as a lower absorbance setting usually caused the Cr-DPC complex peak to be offscale.

2. Effect of Cr(VI) on the DPC and Tris-NaOH Peaks.

A 12cm^3 aliquot of 2 x 10^{-3}M DPC solution was preconcentrated and eluted using the C_{18} column as outlined above. This resulted in the appearance of a peak with an elution time of 18.07 minutes. Next a 12cm^3 aliquot of 0.2M Tris-NaOH solution after adjustment of the pH to 2, using concentrated H_2SO_4, was preconcentrated and eluted as above. This resulted in the appearance of a peak at 8.09 minutes. Following this a mixture of 0.2M Tris-NaOH and DPC was prepared, by adding 2.5cm^3 of 2 x 10^{-3}M DPC to 20cm^3 of 0.2M Tris-NaOH and the solution was adjusted to pH 2 using concentrated H_2SO_4. This solution was then preconcentrated and eluted in the same way. This resulted in the appearance of both of the peaks, which appeared for the DPC and the Tris-NaOH pH 2 (figure 2.14.(a).). Following this a 20cm^3 aliquot of 15ppb Cr(VI) in 0.2M Tris-NaOH was adjusted to pH 2, using concentrated H_2SO_4. A 2.5cm^3 aliquot of DPC was then added and the above preconcentration and elution procedure was carried out again. This experiment resulted again in the appearance of the DPC peak at (7.54 minutes), the Cr(VI)-DPC peak (8.55 minutes)and the 0.2M Tris-NaOH pH 2 peak (18.27 minutes) as shown in figure 2.14.(b). Note that 2.14.(b). is recorded at a lower sensitivity then 2.14.(a). The peak areas of DPC and Tris-NaOH are comparable in both chromatograms. The Cr(VI)-DPC peak is clearly much more intense and less spread out than those of the DPC and Tris-NaOH components. Also the small peak which occurs in the blank (figure 2.14.(a).) at 8.74 minutes would cause negligible error in the measurement of 15ppb Cr(VI).

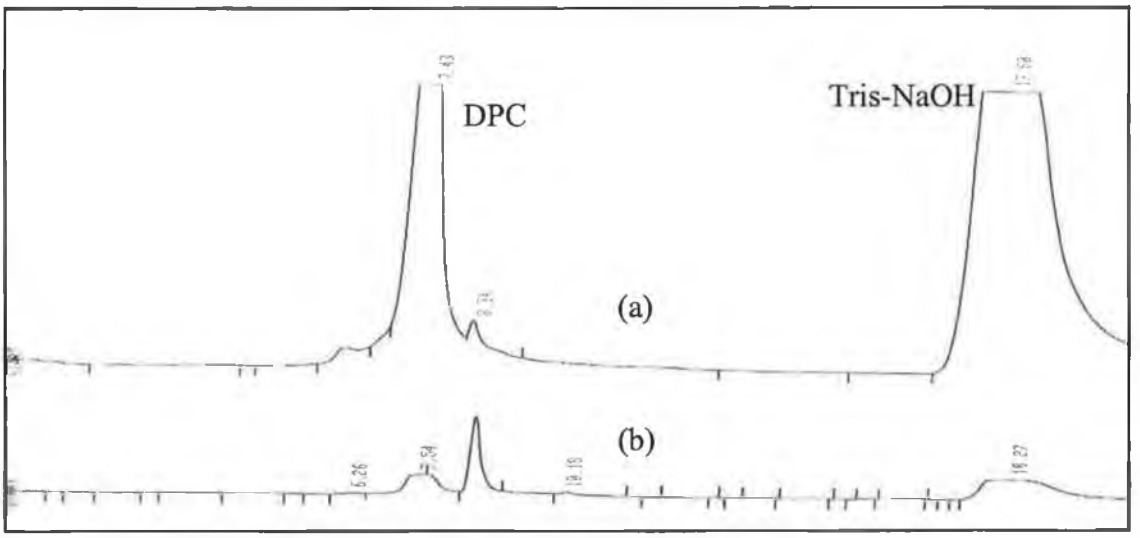


Figure 2.14. Chromatograms of (a) Tris-NaOH and DPC only and (b) Tris-NaOH, DPC and 15ppb Cr(VI).

3. H.P.L.C. of Cr(III).

In order to find out if Cr(III) is preconcentrated onto the column the following experiment was carried out. A 20cm^3 aliquot of 15ppb Cr(III) was prepared and the pH was adjusted to 2 using concentrated H_2SO_4. A 2.5cm^3 aliquot of DPC was then added and the preconcentration and elution procedure was carried out as above. This resulted in the appearance of peaks at 7.5 and 18 minutes only, but these are due to the presence of 0.2M Tris-NaOH and DPC respectively, indicating that the Cr(III) did not adhere to or elute from the column and therefore the excess Cr(III) in the chromium enriched sample will not interfere with Cr(VI) detection using this H.P.L.C. method.

4. Detection of Cr(VI) in Excess Cr(III).

Because the enriched yeast extract contains excess Cr(III) over Cr(VI) it was decided to see if Cr(VI) can be detected in excess Cr(III) using this H.P.L.C. preconcentration and elution method as follows. A 20cm^3 aliquot of each of the following solutions was

prepared and adjusted to pH 2 and 2.5cm^3 of 2 x 10^{-3}M DPC solution was added and the preconcentration and elution procedure set out above was carried out.

(a). 15ppb Cr(VI) and 15ppb Cr(III) in 0.2M Tris-NaOH pH 13.

(b). 15ppb Cr(VI) and 1.5ppm Cr(III) in 0.2M Tris-NaOH pH 13.

(c). 15ppb Cr(VI) and 50ppm Cr(III) in 0.2M Tris-NaOH pH 13.

Result;

For (a) above a peak of area 1700800 eluted at 9.26 minutes, for (b) a peak of area 1715600 eluted at 8.62 minutes and for (c) a peak of area 1695700 eluted at 8.57 minutes. Since the Cr(VI) peak area remained fairly constant as did the elution time it appears that excess Cr(III) has no effect on the preconcentration or elution of Cr(VI). Therefore, Cr(VI) can be detected in excess Cr(III) using this H.P.L.C. preconcentration and elution method and so if there is Cr(VI) present in the chromium enriched yeast sample this procedure will be able to detect it. The retention time of Cr(VI) in this experiment was shorter than that of previous experiments. This may have been due to instrumentation drift or a change in temperature.

5. Preparation of Calibration Curve.

A calibration curve from 0 to 25 ppb Cr(VI) in 5 ppb increments was prepared by applying the above preconcentration and elution procedure to each of the following Cr(VI) standards: 5 ppb, 10ppb, 15ppb, 20ppb and 25ppb. A chromatogram was obtained for each of these Cr(VI) standards which are shown in figure 2.15. It was found that the Cr-DPC complex had a retention time of approximately 11.5 minutes and that as the Cr(VI) concentration increased so too did the area of the Cr-DPC

complex peak. Figure 2.16 represents the linear calibration curve obtained from these chromatograms and the data is given in appendix 2.10.

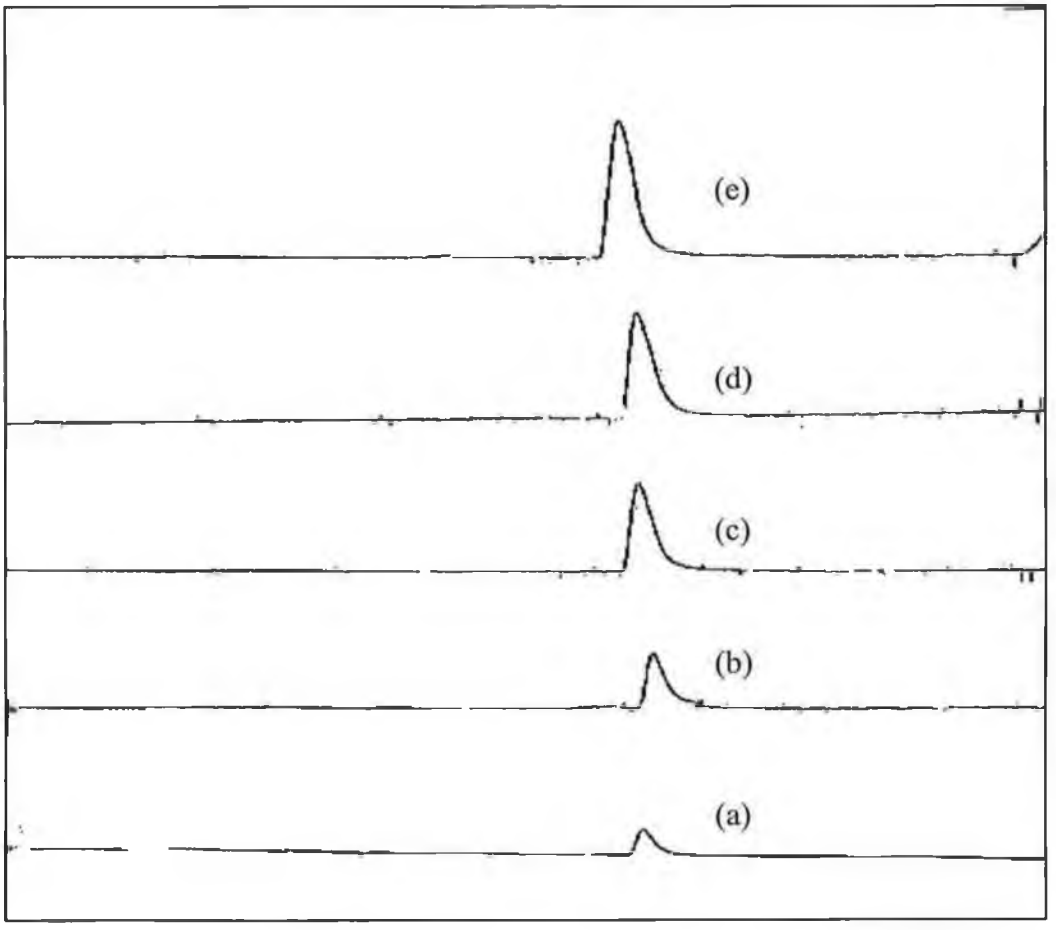


Figure2.15. Chromatograms of the Cr(VI) standards (a) 5ppb, (b) 10ppb, (c) 15ppb, (d) 20ppb and (e) 25ppb Cr(VI).

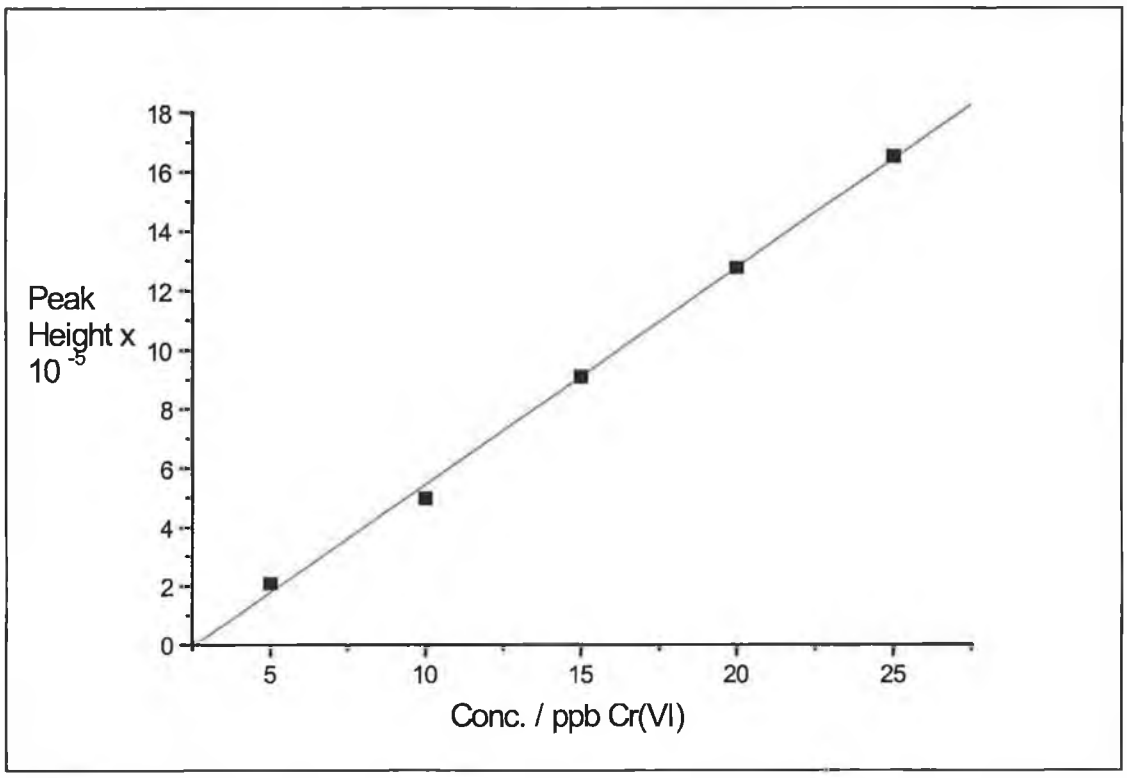


Figure 2.16. Calibration Curve for High Performance Liquid Chromatography.

Table 2.10. Data from calibration curve for H.P.L.C.

Parameters	Value
A	-1.89212±0.3377469
B	0.732772±0.0203669
R	0.99884
SD	0.3220290
N	5
P	0.00005

The limit of detection for this method was calculated as follows;

Limit of Detection = Intercept + 3 (Standard Deviation) = Y value.

$$Y = A + B * X$$

$$\Rightarrow Y - A / B = X$$

X = Limit of Detection.

L.O.D. = 1.89212 + 3 x 0.3220290 = 2.858207

2.858207 – (-1.89212) / 0.732772 = 6.48ppb

2.1.5. Discussion.

The limits of detection, (sample calculation above), of each of the previous mentioned methods for the detection of Cr(VI) were calculated and found to be;

(a). Voltammetry 0.1ppb

(b). G.F.A.A. Spectroscopy 1.36ppb

(c). U.V.Spectroscopy. 43.2ppb

(d). H.P.L.C. 6.5ppb

The voltammetric method was not suitable for the detection of Cr(VI) in the chromium enriched yeast sample because the Cr(III) peak appears at the same potential as that of the Cr(VI) peak and since the chromium enriched yeast sample contains excess Cr(III) it would interfere with the detection of Cr(VI).

The G.F.A.A.spectroscopic method also proved to be unsuitable for the analysis of Cr(VI) in the chromium enriched yeast sample since G.F.A.A. spectroscopy is not specific for Cr(VI), i.e. it measures total chromium, therefore the excess Cr(III) in the enriched yeast sample would interfere with the detection of Cr(VI). Therefore, Cr(III) and Cr(VI) would have to be separated prior to analysis with either of the above described methods.

Since the U.V. spectrophotometric method mentioned here, i.e. the Cr-DPC reaction, is specific for Cr(VI) (Cr(III) is unreactive to DPC) this method of detection of Cr(VI) would be very suitable for the analysis of Cr(VI) in the chromium enriched yeast sample because the presence of the excess Cr(III) would not interfere with Cr(VI) detection. The limit of detection is high compared to the other techniques and therefore preconcentration is necessary.

The H.P.L.C. method developed here would also to be very suitable for the analysis of Cr(VI) in the chromium enriched yeast extract since the C_{18} column used did not retain Cr(III) or organic matter, i.e. it only retained the Cr-DPC complex. Therefore, the excess Cr(III) present in the chromium enriched yeast sample will not interfere with the detection of Cr(VI) and if there is any Cr(VI) present in the sample, this method for the preconcentration and elution of Cr(VI) in the presence of excess Cr(III) will detect it.

The lowest concentration of Cr(VI) that could be detected in the presence of Cr(III) is 6.5ppb. However, the volume of sample used in the preconcentration step was $20cm^3$. Using a larger volume of sample should decrease the limit of detection. Using a $100cm^3$ sample should lower the detection limit to 1.3ppb Cr(VI).

2.2. EXTRACTION OF Cr(VI).

2.2.1. Extraction of Cr(VI) from Yeast using Tris-NaOH in the Presence of Air.

2.2.1.(a). Reagents and Solutions.

1. Tris(hydroxymethyl)aminomethane, $C_4H_{10}NO_3$, F.W.=121.14g mol^{-1}, Riedel-de Haen, #33742.
2. Chromium(III) Chloride, $CrCl_3.6H_2O$, F.W. = 266.45g mol^{-1}, Riedel-de Haen, #12228.
3. Potassium Dichromate $K_2Cr_2O_7$, F.W. = 294.19g mol^{-1}, Wardle Chemical Company Ltd., #PO4868.
4. Chromium Enriched Yeast, Alltech Ireland, Dunboyne, Co. Meath, Ref. 158233.
5. Non-Enriched Yeast, Active Dried Yeast, DCL Yeast Limited.
6. 1,5-Diphenylcarbazide, $C_{13}H_{14}N_4O$, F.W.=242.3g mol^{-1}, Aldrich Chemical Company Inc., #25922-5.

Preparation of Solutions.

1. *0.2M Tris-NaOH pH 13.*

   As in section 2.1.1.(b).

2. *10ppm Cr(VI).*

   As in section 2.1.3.(b).

3. *1.67 x 10^{-3} M DPC, 0.68M H_2SO_4.*

   40cm^3 of concentrated H_2SO_4 were diluted to 400cm^3 with doubly distilled water. 0.2027g of 1,5-diphenylcarbazide were dissolved in 100cm^3 of doubly distilled water. These two solutions were mixed with constant stirring.

2.2.1.(b). Apparatus.

1. Balance - Mettler AE 160, Supplied by Mason Technology, Dublin.
2. Centrifuge - Sigma - 302K.
3. 0.45µm Cellulose Acetate Filter, 47mm, 0.45µm, Gelman Sciences, Lot No. 63172.
4. Polycarbonate Filter Holder, Sortorius.
5. Magnetic Stirrer - Bibby HB 501.

2.2.1.(c). Extraction Procedure carried out in the Presence of Air.

Flores-Velez et al (1994) used Tris-NaOH pH 13 buffer for the extraction of Cr(VI) from soils and found that it was capable of extracting up to 98.6+/-2.2% of added Cr(VI). The buffer has the advantage that it will extract sparingly soluble Cr(VI) species. Also Cr(VI) is a weak oxidising agent in alkaline solution and will persist longer than it will in more acidic extraction buffers.

1. The Extraction Procedure in the Presence of Air.

A 1g sample of yeast was added to $50cm^3$ of 0.2M Tris-NaOH pH 13, and immediately agitation of the solution commenced. After 24 hours of stirring, this solution was centrifuged for 30 minutes at a rate of 8000 rpm^{-1}. The solution was then filtered through a 0.45µm cellulose acetate filter using a polycarbonate filter holder. The filtrate was then made up to $100cm^3$ using 0.2M Tris-NaOH pH 13 and stored in a $100cm^3$ volumetric flask. To a $5cm^3$ aliquot of this extracted solution a $2.5cm^3$ aliquot of DPC solution was added and made up to $25cm^3$ using doubly distilled water. After 5 minutes (the time taken for the formation of the Cr(VI)-DPC complex to form) the solution was analysed using the UV spectrophotometric method outlined

in section 2.1.3.(d). Every 24 hours following this, a $5cm^3$ aliquot of the extracted solution was analysed in this way for a total of 9 days.

2. To show that Chromium(VI) can be Extracted in the Presence of Yeast.

To show that the above extraction procedure succeeded in extracting chromium(VI) from the chromium enriched yeast, extraction of chromium from the following synthetic samples where chromium(III) is added to non-enriched yeast to simulate the presence of excess Cr(III) in the chromium enriched yeast and chromium(VI) was added at zero, 0.5 and 1ppm levels. The samples were

(A). Non-Enriched Yeast + 20ppm Cr(III).

(B). Non-Enriched Yeast + 20ppm Cr(III) + 500ppb Cr(VI).

(C). Non-Enriched Yeast + 20ppm Cr(III) + 1ppm Cr(VI).

Samples were prepared using the quantities outlined in Table 2.11 where the Cr(III) and Cr(VI) were added to the solution after the 1g of yeast was added to $50cm^3$ of 0.2M Tris-NaOH pH 13.

Table 2.11. Quantities required to produce the above solutions.

	Mass of Non-Enriched yeast / g	Mass of $CrCl_3.6H_2O$ / g	Volume of 10ppm Cr(VI) / cm^3
(A)	1.0015	0.0103	–
(B)	1.0015	0.0103	5
(C)	1.0012	0.0103	10

After the extraction was carried out $5cm^3$ of the extracted solution was analysed every 24 hours for 9 days using the UV spectrophotometric method outlined in section 2.1.3.(d). These experiments resulted in all 3 extractions giving an absorbance

reading on day 1 and these readings increased over time. Extract A, which contains no added Cr(VI), gave an absorbance reading on day 1. This implies that air oxidation of Cr(III) occurs in the extraction process. Extract B and C gave significantly higher Cr(VI) readings on day 1 than extract A. The absorbance was not directly proportional to the concentration of added Cr(VI). This indicates that some reduction of Cr(VI) occurs initially in these experiments, followed by air oxidation. Figure 2.17 represents the absorbance readings obtained for the extraction of these simulated chromium enriched yeast samples and the data is given in appendix 2.11.

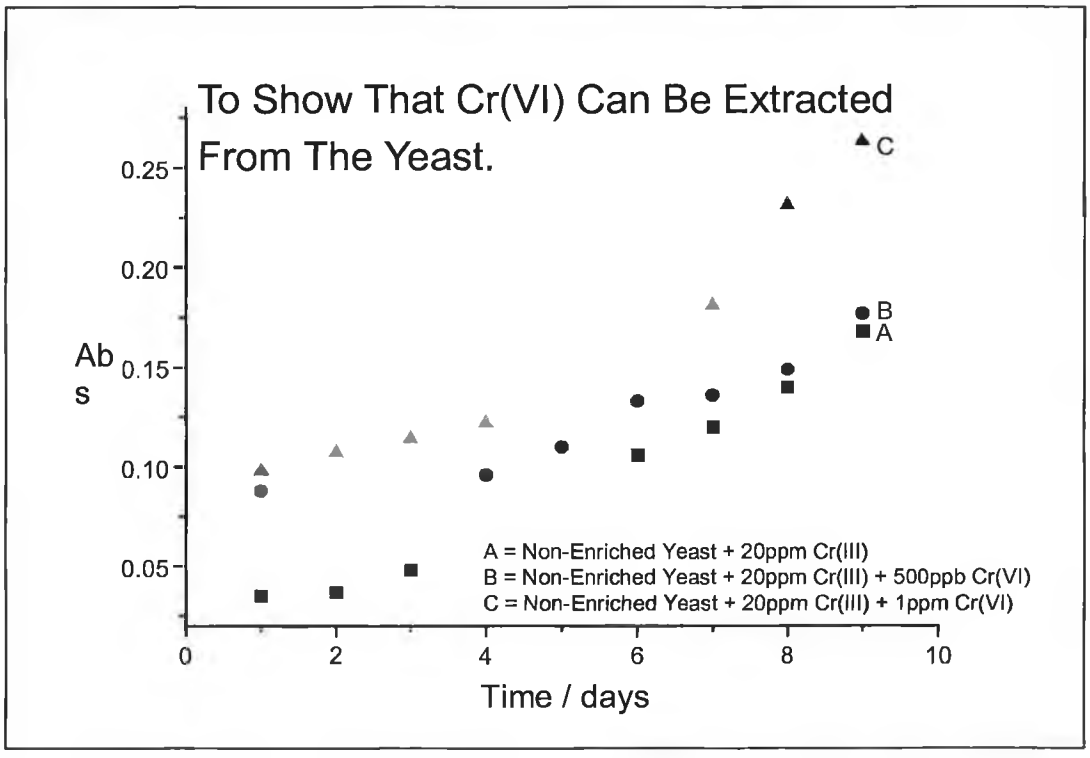


Figure 2.17. To show that chromium(VI) can be extracted from the simulated chromium enriched yeast.

3. To Show that Cr(VI) can be Extracted in the Presence of Chromium Enriched Yeast.

Since the absorbance readings of all 3 extracts in the previous experiment increased over time it was suspected that air oxidation of Cr(III) to Cr(VI) had occurred, which was a factor that was not normally previously considered in chromium speciation studies. In order to find out if this oxidation process also occurred in the presence of chromium enriched yeast the extraction procedure above was carried out on the following solutions;

(A). Chromium enriched yeast + 500ppb Cr(VI) + 20ppm Cr(III).

(B). Chromium enriched yeast + 500ppb Cr(VI).

using the quantities listed in Table 2.12 below by adding the Cr(III) and Cr(VI) to the solution after the chromium enriched yeast was added to the Tris-NaOH buffer. A $5cm^3$ aliquot of the extracted solution was then analysed every 24 hours for 9 days using the spectrophotometric method in section 2.1.3.(d).

Table 2.12. Quantities required to produce the above solutions.

	Mass of Chromium Enriched Yeast / g	Mass of $CrCl_3.6H_2O$ / g	Volume of 10ppm Cr(VI) / cm^3
(A)	1.0031	0.0102	5
(B)	1.0024	-	5

These extraction procedures resulted in both of the solutions giving an absorbance measurement on day 1 and both these readings increased over time. Solution A, which contained added Cr(III), gave higher absorbance measurements than solution B, which contained no added Cr(III), thus implying that air oxidation of the added Cr(III) to Cr(VI) had occurred more rapidly than that of the bound Cr(III). The rate of

increase of the Cr(VI) concentration in both cases was similar. Figure 2.18 represents the absorbance readings obtained for the extraction of these solutions and the data is given in appendix 2.12.

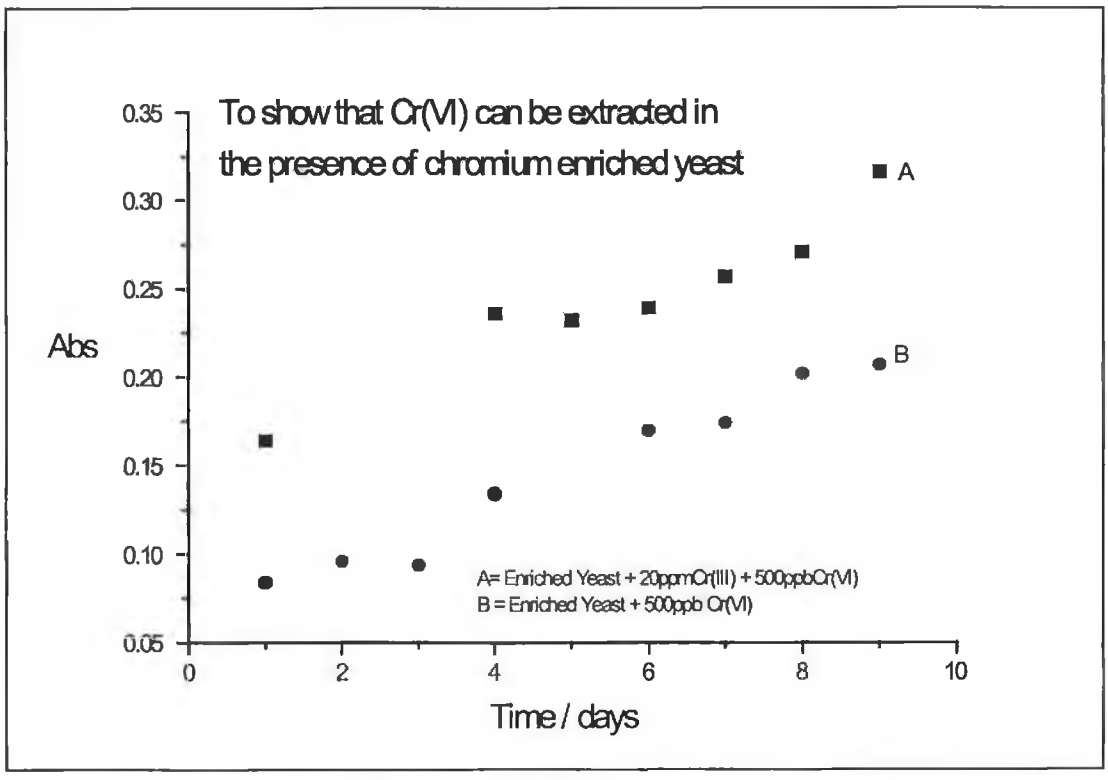


Figure 2.18. To show air oxidation of Cr(III) to Cr(VI) in alkaline solution.

2.2.2. Extraction of Cr(VI) from Yeast using Tris-NaOH in the Absence of Air (i.e. under Nitrogen).

2.2.2.(a). Reagents and Solutions.

1. Tris(hydroxymethyl)aminomethane, $C_4H_{10}NO_3$F.W.=121.14g mol^{-1}, Riedel-de Haen, #33742.
2. Chromium (III) Chloride,$CrCl_3.6H_2O$, F.W.=266.45g mol^{-1}, Riedel-de Haen, #12228.
3. Potassium Dichromate, $K_2Cr_2O_7$, F.W.=294.19g mol^{-1}, Wardle Chemical Company Ltd., #PO4868.
4. Chromium Enriched Yeast, Alltech Ireland, Dunboyne, Co. Meath, Ref. 158233.
5. Non-Enriched Yeast, Active Dried Yeast, DCL Yeast Limited.
6. 1,5-Diphenylcarbazide, $C_{13}H_{14}N_4O$, F.W.=242.3g mol^{-1}, Aldrich Chemical Company Inc., #25922-5.

Preparation of Solutions.

1. *0.2M Tris-NaOH pH 13.*

   As in section 2.1.1.(b).

2. *1.67 x 10^{-3} M DPC.*

   As in section 2.2.1.(a).

3. *10ppm Cr(VI).*

   As in section 2.1.3.(b).

2.2.2.(b). Apparatus.

1. Balance - Mettler AE 160, Supplied by Mason Technology, Dublin.
2. Centrifuge - Sigma - 302K.

3. 0.45µm Cellulose Acetate Filter, 47mm, 0.45µm, Gelman Sciences, Lot No. 63172.

4. Polycarbonate Filter Holder, Sortorius.

5. Magnetic Stirrer - Bibby HB 501.

6. Nitrogen Cyclinder (Oxygen Free) BOC Ltd..

7. Parafilm - American National Can. PM-992.

2.2.2.(c). Extraction Procedure.

Since the experiments in the previous section show that in air Cr(III) can be oxidised to Cr(VI) it was decided to carry out the extraction process in the absence of air.

1. The Extraction Procedure carried out in this section was as follows.

A 50cm^3 aliquot of 0.2M Tris-NaOH pH 13 were de-aerated with nitrogen for 15 minutes. A 1g sample of yeast was added to this de-aerated Tris-NaOH and the solution was de-aerated again for 15 minutes. Agitation of the solution commenced immediately and the solution was left stirring, sealed and under nitrogen. After 24 hours the solution was poured into the centrifuge tube and de-aerated while in the tube, sealed with parafilm and centrifuged for 30 minutes at a rate of 8000rpm^{-1}. The solution was filtered, under nitrogen, through a 0.45µm cellulose acetate filter using a polycarbonate filter holder. The filtrate was then made up to 100cm^3 with de-aerated 0.2M Tris-NaOH pH 13 and stored in a round bottomed flask under nitrogen. To 5cm^3 of the extracted solution 2.5cm^3 of DPC solution were added and made up to 25 cm^3 with doubly distilled water. After 5 minutes (the time taken for the formation of the Cr(VI)-DPC complex) the solution was analysed using the UV spectrophotometric method in section 2.1.3.(d). Every 24 hours following this, 5cm^3 aliquots of this extracted solution were analysed in this way for a total of 9 days.

2. To Show Air Oxidation of Cr(III) To Cr(VI) in Alkaline Solution.

A 0.0102g sample of $CrCl_3.6H_2O$ was dissolved in $50cm^3$ of deaerated 0.2M Tris-NaOH pH 13. Immediately a $5cm^3$ aliquot of this solution was withdrawn, a $2.5cm^3$ aliquot of DPC solution was added and the solution was diluted to $25cm^3$ with doubly distilled water. Analysis was then carried out at 540nm. The remainder of the solution was left stirring with nitrogen over it. A $5cm^3$ aliquot of this solution was withdrawn and analysed as above on a daily basis. It was found that no absorbance measurements were recorded on day 1 or on any of the 9 days following this.

A 0.0102g sample of $CrCl_3.6H_2O$ was also added to $50cm^3$ of 0.2M Tris-NaOH pH 13. Again a $5cm^3$ aliquots was withdrawn immediately and analysed at 540nm after the addition of a $2.5cm^3$ aliquot of DPC and dilution to $25cm^3$ with doubly distilled water. The remainder of the solution was stirred by bubbling air (saturated with 0.2M Tris-NaOH pH 13) through the solution. It was found that no absorbance was recorded on day 1, but on day 2 an absorbance was recorded and this measurement increased over time. Since an absorbance measurement was recorded for the solution stirred in air and no measurement was recorded for the solution stirred under nitrogen it implies that air oxidation of Cr(III) to Cr(VI) did occur in alkaline solution. Figure 2.19 represents the absorbance measurements obtained for these extractions and the data is given in appendix 2.13.

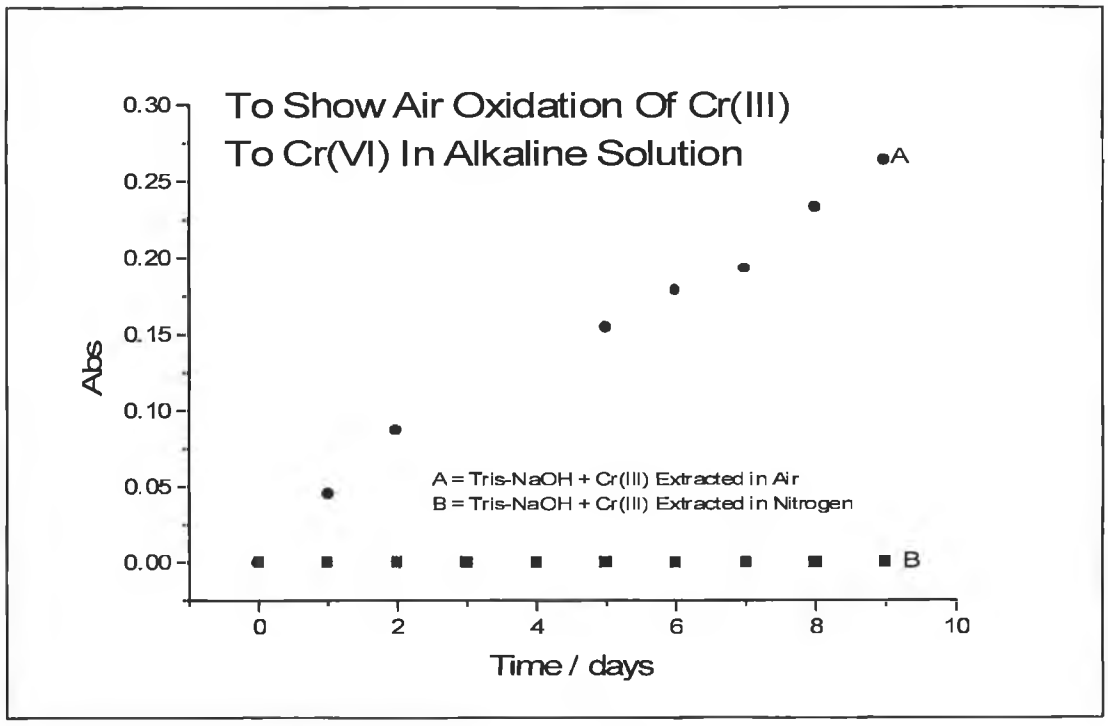


Figure 2.19. To show air oxidation of Cr(III) to Cr(VI) in alkaline solution.

3. To Show That The Chromium Enriched Yeast Reduces Cr(VI) In Inert Atmosphere.

The above extraction procedure was carried out on the chromium enriched yeast plus 500ppb Cr(VI) by adding a $5cm^3$ aliquot of 10ppm Cr(VI) to 1.0005g of the chromium enriched yeast, after it was added to the de-aerated Tris-NaOH buffer, and prior to the second de-aeration step. A $5cm^3$ aliquot of this extracted solution was then analysed every 24 hours for 9 days resulting in no absorbance being recorded on day 1 or on any of the 9 days following. The extraction procedure in air was also carried out on 1.0003g of the chromium enriched yeast plus 500ppb Cr(VI) as described in section 2.2.1.(c). Again $5cm^3$ aliquots were analysed every 24 hours for a total of 9 days. This resulted in an absorbance being recorded on day 1 and this absorbance increased over time. Figure 2.20 represents the absorbance obtained for these extractions and the data is given in appendix 2.14. Since no absorbance was recorded for the extraction carried out under nitrogen, it implies that the chromium enriched

yeast rapidly reduced the Cr(VI) that was added to it, and since a measurement was recorded for the extraction of the same solution in the presence of air it indicates that the chromium enriched yeast is capable of reducing Cr(VI) in inert atmosphere, in basic solution.

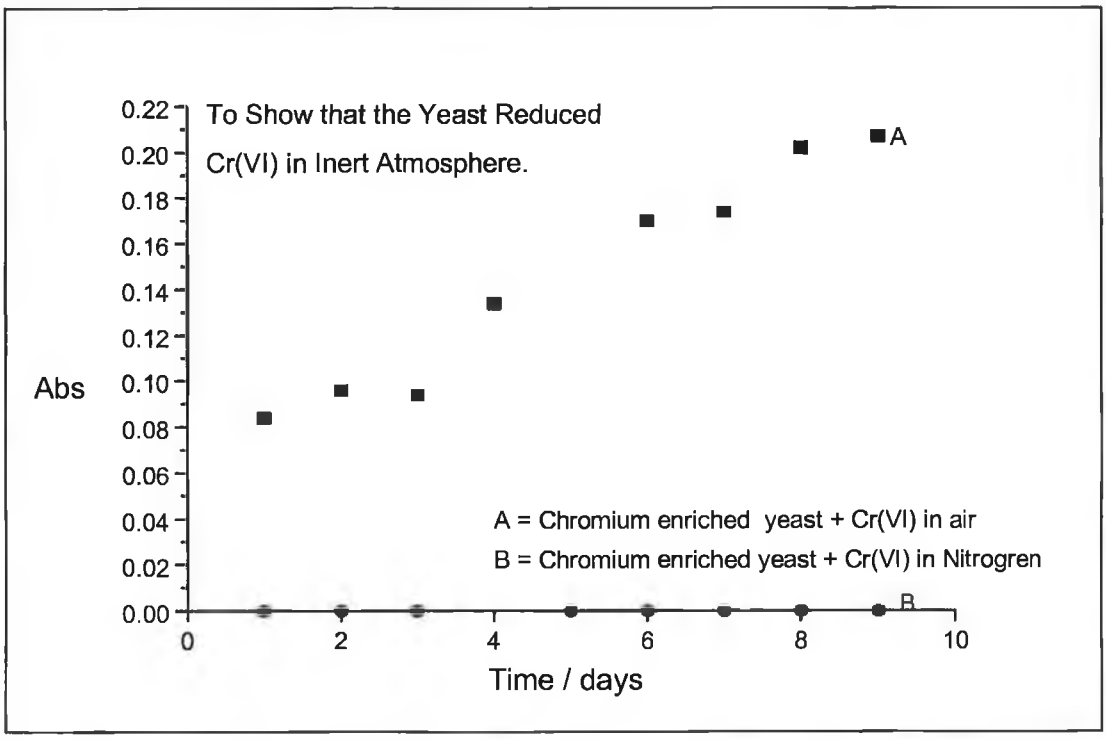


Figure 2.20. To show that the chromium enriched yeast reduced Cr(VI) in inert atmosphere.

4. To Show Air Oxidation Of The Chromium Enriched Yeast Extract.

The above extraction procedure was carried out on 1.0002g of the chromium enriched yeast and $5cm^3$ of the extracted solution were analysed every 24 hours for a total of 9 days. Again this extraction prodecure resulted in no absorbance measurement being recorded over the 9 days. The extraction procedure in air was carried out on 1.0005g of the chromium enriched yeast as described in section 2.2.1.(c). Again $5cm^3$ of this extracted solution were analysed every 24 hours for a total of 9 days resulting in no

absorbance being recorded on day 1 but an absorbance was recorded on day 2 and this absorbance increased over time. Figure 2.21 represents the absorbance obtained for these extractions and the data is given in appendix 2.15. Since no absorbance was recorded for the extraction procedure carried out under nitrogen, and an absorbance was recorded for the extraction of the same solution carried out in the presence of air it indicates that air oxidation of the chromium enriched yeast extract can occur, and that by carrying out the extraction process under nitrogen this oxidation of Cr(III) to Cr(VI) can be eliminated.

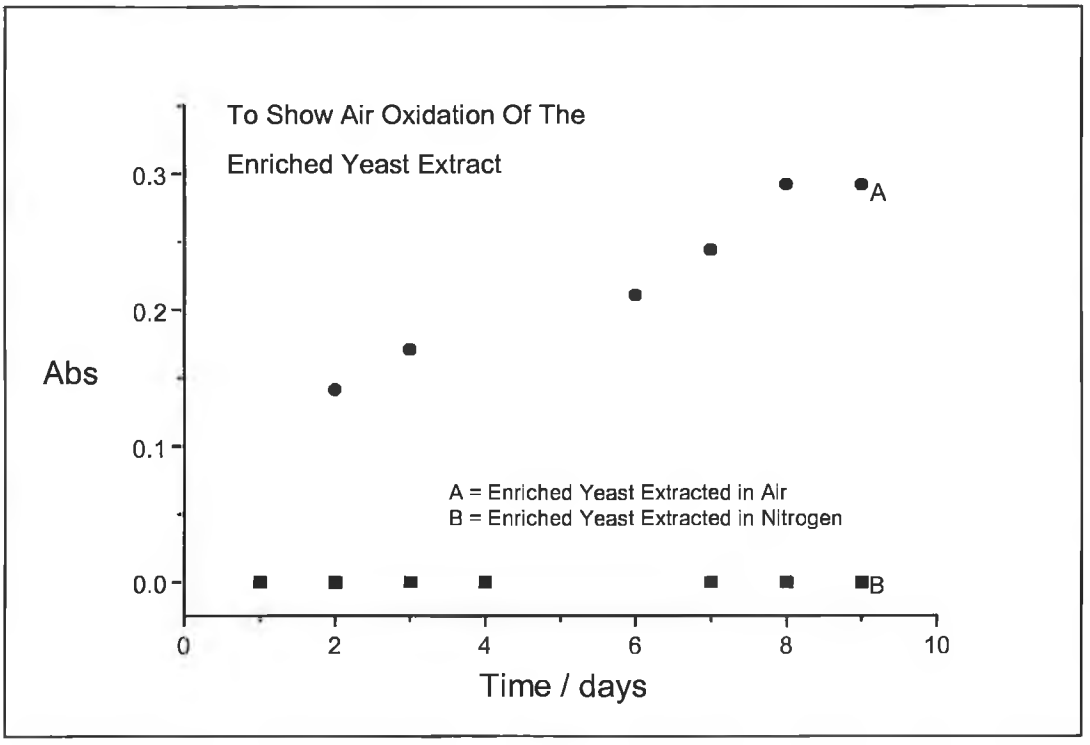


Figure 2.21. To show air oxidation of the chromium enriched yeast.

2.2.3. Effect of leaving the Chromium Enriched Yeast in Contact with the Cr(VI) Solution.

In the above experiments it was shown how air oxidation of Cr(III) to Cr(VI) can occur in a solution of Cr(III) exposed to air at pH 13 and in the chromium enriched yeast extract at pH 13. Therefore it was decided to see if this oxidation process would occur if the chromium enriched yeast was left in contact with the Cr(VI) solution since it was also shown above how the chromium enriched yeast can reduced Cr(VI) in inert atmosphere.

1. Procedure carried out in Air.

A 1g sample of the chromium enriched yeast was added to $50cm^3$ of the 0.2M Tris-NaOH pH 13 buffer. Immediately a $5cm^3$ aliquot of this solution was withdrawn by pipette and filtered through a 0.2μM syringe filter. A $2cm^3$ aliquot of this filtered solution was pipetted into a $10cm^3$ volumetric flask, a $1cm^3$ aliquot of 1.67 $x10^{-3}$M DPC was added and the solution was made up to the mark with doubly distilled water. After 5 minutes a spectrum of this solution was obtained from 800 to 400nm. The remainder of the solution, containing the chromium enriched yeast, was left with air (saturated with 0.2M Tris-NaOH pH 13) bubbling through it. Every 24 hours following this a $5cm^3$ aliquot of this solution was withdrawn, filtered and analysed as above.

This procedure was applied to

(i). 1.0003g of chromium enriched yeast

(ii). 1.0012g of chromium enriched yeast + 1ppm Cr(VI)

The 1ppm Cr(VI) was added to (ii) above by addition of a $5cm^3$ aliquot of 10ppm Cr(VI) to the $50cm^3$ of Tris-NaOH pH 13 after the 1g of chromium enriched yeast had been added.

The results of these experiments are shown in figures 2.22.(a) and 2.22.(b). From figure 2.22.(a) it can be seen that initially there was no Cr(VI) detected, but on day 2 a small concentration of Cr(VI) was detectable and this concentration increased on day 5. But on day 6, 7 and 8 the concentration of Cr(VI) decreased, implying that initially there was air oxidation of the Cr(III) in the yeast but the chromium enriched yeast then reduced this oxidised Cr(VI). Figure 2.22.(b) shows a small concentration of Cr(VI) on day 0. This absorbance reading was not observed in the previous experiment because there was no added Cr(VI) in the solution. This concentration slowly increases over day 1, 2, and 5 but on day 6 the concentration of Cr(VI) present had decreased and this concentration decreased further on day 7 again implying initial air oxidation of the Cr(III) in the yeast and then reduction of this oxidised Cr(VI) by the chromium enriched yeast.

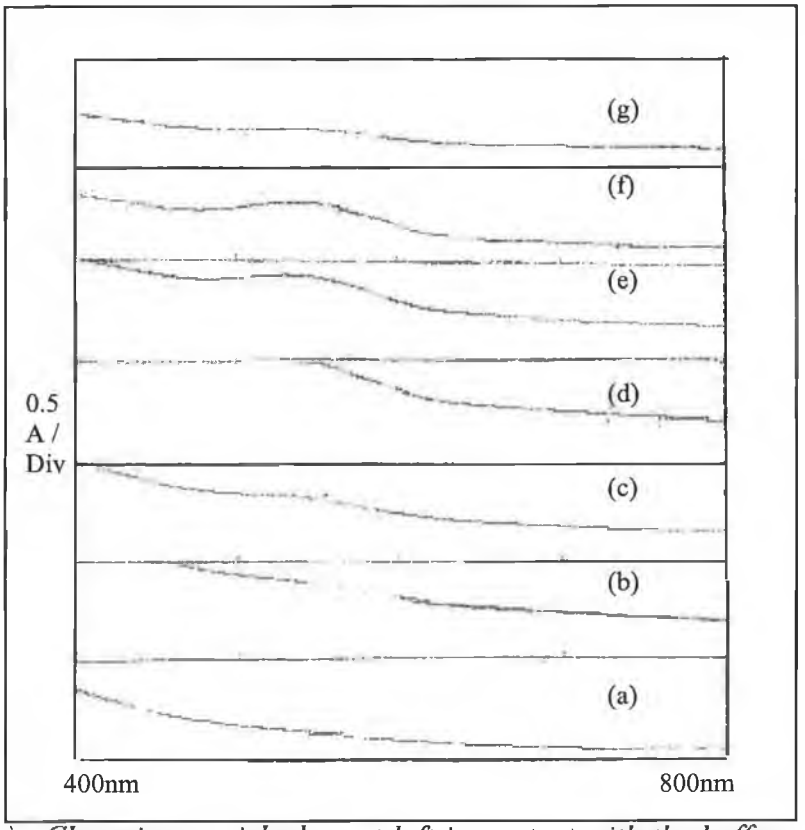


Figure 2.22.(a). Chromium enriched yeast left in contact with the buffer after (a) 0, (b) 1, (c) 2, (d) 5, (e) 6, (f) 7, (g) 8 days.

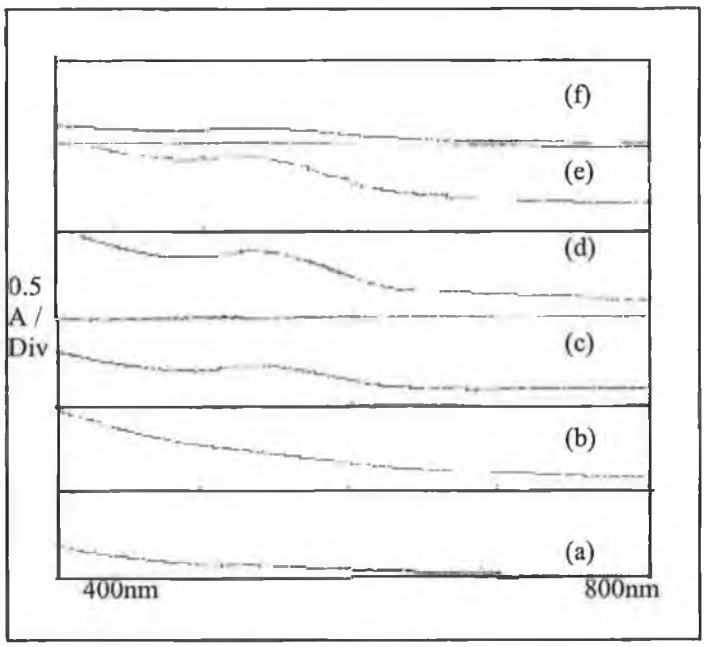


Figure 2.22.(b). Chromium enriched yeast + Cr(VI) left in contact with the buffer after (a) 0, (b) 1, (c) 4, (d) 5, (e) 6, (f) 7 days.

2. Procedure carried out in Nitrogen.

A 50cm^3 aliquot of 0.2M Tris-NaOH pH 13 was deaerated with nitrogen for 15 minutes. A 1g sample of chromium enriched yeast was added to this deaerated solution and immediately a 5cm^3 aliquot was withdrawn by pipette and filtered through a 0.2μM syringe filter. A 2cm^3 aliquot of this solution was then pipetted into a 10cm^3 volumetric flask, a 1cm^3 aliquot of 1.67 x 10^{-3}M DPC was added and the solution was made up to the mark with doubly distilled water. After 5 minutes a spectrum of this solution was obtained from 800nm to 400nm. The remainder of the solution containing the chromium enriched yeast was left stirring with nitrogen over it. A 5cm^3 of this solution was withdrawn, filtered and analysed on a daily basis. This procedure was carried out on the following solution;

(i). 0.9997g of chromium enriched yeast

(ii). 1.0005g of chromium enriched yeast + 1ppm Cr(VI)

The 1ppm Cr(VI) was added to (ii) above by addition of a deaerated $5cm^3$ aliquot of 10ppm Cr(VI) to the $50cm^3$ of deaerated Tris-NaOH pH 13 after the 1g of chromium enriched yeast had been added. The results of these experiments are shown in figure 2.23.(a) and 2.23.(b) respectively.

In figure 2.23.(a) it can be seen that on day 0 no Cr(VI) was detected. This agrees with the experiment 4 in section 2.2.2.(c) which shows that oxidation of the chromium enriched yeast extract does not occur in inert atmosphere. But on day 1 Cr(VI) was detected and this concentration of Cr(VI) increased on day 4, 5 and 6 and on day 7 the concentration of Cr(VI) decreased dramatically.

Figure 2.23.(b) shows that on day 0 and 1 no Cr(VI) was detected implying that the chromium enriched yeast reduced the added Cr(VI), which agrees with the results obtained in experiment 3 in section 2.2.2.(c). On day 2 Cr(VI) was detected and the concentration increased on day 5, decreased on day 6 and increased again on day 7. These results were unexpected as it had been shown previously that under a nitrogen atmosphere Cr(III) would not be oxidised to Cr(VI) over several days. These experiments are discussed later, section 3.5.5 (page 235).

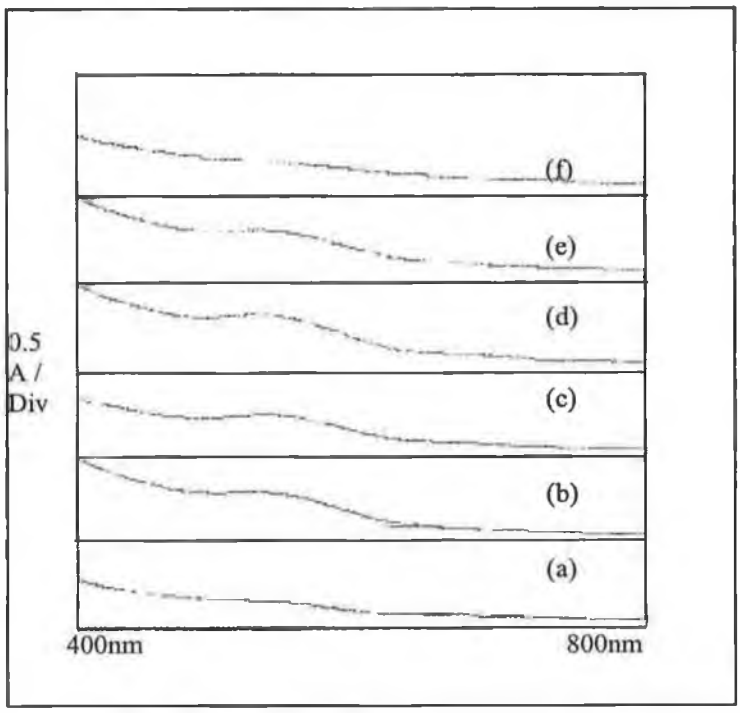


Figure 2.23.(a). Chromium enriched yeast left in contact with the buffer under nitrogen after (a) 0, (b) 1, (c) 4, (d) 5, (e) 6, (f) 7 days.

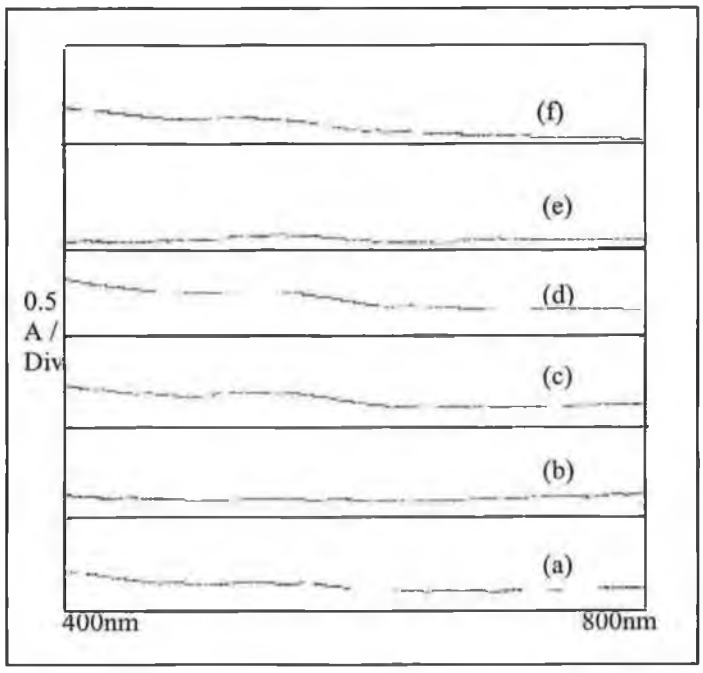


Figure 2.23.(b). Chromium enriched yeast + Cr(VI) left in contact with the buffer under nitrogen after (a) 0, (b) 1, (c) 4, (d) 5, (e) 6, (f) 7days.

2.3. PRECONCENTRATION OF Cr(VI) BY ION EXCHANGE.

2.3.1. Reagents and Solutions.

1. Ammonia, NH_3, F.W.=17.03g mol^{-1}, BDH GPR, # 27141.
2. Tris(hydroxymethyl)aminomethane, $C_4H_{10}NO_3$, F.W.=121.14g mol^{-1}, Riedel-de Haen, #33742.
3. Potassium Dichromate, $K_2Cr_2O_7$, F.W. = 294.19g mol^{-1}, Wardle Chemical Company Ltd., #PO4868.
4. Amberlite IRA - 410, Ionic Form Chloride, Strongly Basic Anion Exchanger, Sigma Chemical Company, Lot No. 87H0670.
5. Ammonium Sulphate, $(NH_4)_2SO_4$, F.W.=132.13g mol^{-1}, Mallinckrodt Analytical Reagent.
6. 1,5-Diphenylcarbazide, $C_{13}H_{14}N_4O$, F.W.= 242.3g mol^{-1}, Aldrich Chemical Company Inc., #25922-5.
7. Sulphuric Acid, H_2SO_4, F.W.=98.07g mol^{-1}, Riedel-de Haen, UN-No. 1830.
8. L-Ascorbic Acid, $C_6O_6H_8$, F.W.=176.13 g mol^{-1}, BDH Biochemicals Ltd., # 44006.
9. Ultra Pure Nitric Acid, HNO_3, F.W.=63.01g mol^{-1}, Aldrich Chemical Company Inc., #25812-1.
10. Chromium Enriched Yeast, Alltech Ireland, Dunboyne, Co. Meath, Ref. 158233.
11. Non Enriched Yeast, Active Dried Yeast, DCL Yeast Limited.
12. Chromium(III) Chloride, $CrCl_3.6H_2O$, F.W.=266.45g mol^{-1}, Riedel-de Haen, #12228.

Preparation of Solutions.

1. *0.2M HN_4OH pH 10.*

   13.5cm^3 of ammonia were diluted to 1 dm^3 with distilled water and the pH was adjusted to 10 using concentrated ammonia.

2. *0.2M Tris-NaOH pH 13.*

   As in section 2.1.1.(b).

3. *Cr(VI) Standards.*

   (a). 1000ppm Cr(VI) in Tris-NaOH pH 13.

   A mass of 0.2829g of potassium dichromate was dissolved in 100cm^3 of 0.2M Tris-NaOH pH 13.

   (b). 10ppm Cr(VI) in Tris-NaOH pH 13.

   A 1cm^3 aliquot of 1000ppm Cr(VI) was diluted to 100cm^3 using 0.2M Tris-NaOH pH 13.

   (c). 1ppm Cr(VI) in Tris-NaOH pH 13.

   A 10cm^3 aliquot of 10ppm Cr(VI) was diluted to 100cm^3 using 0.2M Tris-NaOH pH 13.

   (d). 100ppb Cr(VI) in Tris-NaOH pH 13.

   A 1cm^3 aliquot of 10ppm Cr(VI) was diluted to 100cm^3 using 0.2M Tris-NaOH pH 13.

   (e). 10ppb Cr(VI) in Tris-NaOH pH 13.

   A 1cm^3 aliquot of 1ppm Cr(VI) was diluted to 100cm^3 using 0.2M Tris-NaOH pH 13.

   (f). 1ppb Cr(VI) in Tris-NaOH pH 13.

   A 1cm^3 aliquot of 100ppb Cr(VI) was diluted to 100cm^3 using 0.2M Tris-NaOH pH 13.

(g). 5ppb Cr(VI) in Tris-NaOH pH 13.

A 5cm^3 aliquot of 100ppb Cr(VI) was diluted to 100cm^3 using 0.2M Tris-NaOH pH 13.

4. *2M $(NH_4)_2SO_4$, 4M NH_4OH pH 10.*

   26.4263g of ammonium sulphate were dissolved in 50cm^3 of distilled water. 26cm^3 of ammonia were added and this solution was made up to 100cm^3 with distilled water. The pH was adjusted to 10 using concentrated nitric acid.

5. *2M $(NH_4)_2SO_4$, 2M NH_4OH pH 10.*

   26.4263g of ammonia sulphate were dissolved in 50cm^3 of distilled water. 13cm^3 of ammonia were added and this solution was made up to 100cm^3 with distilled water. The pH was adjusted to 10 using concentrated nitric acid.

6. *2M $(NH_4)_2SO_4$, 0.8M NH_4OH pH 10.*

   26.4263g of ammonia sulphate were dissolved in 50cm^3 of distilled water. 5.2cm^3 of ammonia were added and this solution was made up to 100cm^3 with distilled water. The pH was adjusted to 10 using concentrated nitric acid.

7. *2M $(NH_4)_2SO_4$, 8.0M NH_4OH pH 10.*

   26.4263g of ammonia sulphate were dissolved in 30cm^3 of distilled water. 52cm^3 of ammonia were added and this solution was made up to 100cm^3 with distilled water. The pH was adjusted to 10 using concentrated nitric acid.

8. *2M $(NH_4)_2SO_4$, 8.0M NH_4OH pH 11.9.*

   26.4263g of ammonia sulphate were dissolved in 30cm^3 of distilled water. 52cm^3 of ammonia were added and this solution was made up to 100cm^3 with distilled water.

9. 2M $(NH_4)_2SO_4$, 4.0M NH_4OH pH 9.0.

26.4263g of ammonia sulphate were dissolved in 50cm^3 of distilled water. 26cm^3 of ammonia were added and this solution was made up to 100cm^3 with distilled water. The pH was adjusted to 9.0 using concentrated nitric acid.

10. 1,5-Diphenylcarbazide Solution.

As in section 2.1.3.(b).

11. 1.45M H_2SO_4.

8cm^3 of concentrated H_2SO_4 were diluted to 100cm^3 using distilled water.

12. Anion Exchange Columns.

(a). Long Columns.

15g of amberlite IRA-410 were weighed and excess distilled water was added. The ion exchange column was half filled with distilled water and with the aid of a funnel the well stirred slurry of resin was transferred to the ion exchange column until a height of 18cm was reached. The column was allowed to drain to 1cm from the top of the resin and this excess was maintained throughout.

(b). Short Column.

This was prepared in the same way as the long column with the exception that only 7g of amberlite IRA-410 was weighed and the column was packed to a height of 7cm.

13. 0.1M H_2SO_4, 0.1M Ascorbic Acid.

1M Ascorbic Acid.

17.6131g of ascorbic acid were dissolved in 100cm^3 of distilled water.

2.7cm^3 of H_2SO_4 were measured, some distilled water was added, 50cm^3 of the 1M Ascorbic Acid were added and this solution was diluted to 500cm^3 with distilled water.

14. Cr(VI) Standards.

As in section 2.1.2.(b).

15. 0.1M H_2SO_4.

2.7cm^3 of concentrated H_2SO_4 were diluted to 500cm^3 with distilled water.

16. Cr(III) Standards in 0.2M Tris-NaOH pH 13.

(a). 1000ppm Cr(III) in 0.2M Tris-NaOH pH 13.

0.5128g of $CrCl_3.6H_2O$ were dissolved in 100cm^3 of 0.2M Tris-NaOH pH 13.

(b). 50 ppm Cr(III) in 0.2M Tris-NaOH pH 13.

A 5cm^3 aliquot of 1000ppm Cr(III) in 0.2M Tris-NaOH pH 13 was diluted to 100cm^3 with 0.2M Tris-NaOH pH13.

17. 50ppm Cr(III) and 100ppb Cr(VI) in 0.2M Tris-NaOH pH 13.

A 1cm^3 aliquot of 1000ppm Cr(VI) in 0.2M Tris-NaOH pH 13 was diluted to 100cm^3 with 0.2M Tris-NaOH pH13. A 1cm^3 aliquot of this was then measured, 5cm^3 of 1000ppm Cr(III) in 0.2M Tris-NaOH were added and the solution was diluted to 100cm^3 again with 0.2M Tris-NaOH pH 13.

2.3.2. Instrumentation.

(a). UV-160, UV-Visible recording Spectrophotometer, Shimadzu.

(b). Spectra AA-10 Atomic Absorption Spectrometer.

Varian GTA-96 Graphite tube Atomiser.

Chromium Hollow Cathode Lamp, Pye Unicam Ltd., #39804.

Seikosha SL-SOIP Printer.

Sample Holders, JVA Analytical Ltd..

2.3.3. Preconcentration Procedure using U.V. Spectrophotometric Detection.

An ion exchange column was prepared as in section 2.3.1. and conditioned with 100cm^3 of 0.2M NH_4OH pH 10. The solution to be analysed was next applied to the column and then the column was washed with 25cm^3 of 0.2M Tris-NaOH pH 13. The Cr(VI) which adhered to the column was then eluted using 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 10. A flow rate of 6 drops per 10 seconds was retained throughout. A 3cm^3 fraction was collected manually every minute, a 2.0cm^3 aliquot of 1.67 x 10^{-3}M DPC was added to each fraction and after the Cr(VI)-DPC complex formed the absorbance measurements were obtained at 540nm using the spectrophotometric method outline in section 2.1.3.(d).

2.3.4. Preconcentration Procedure using G.F.A.A.S.

Again an anion exchange column was prepared as in section 2.3.1. and conditioned with 100cm^3 of 0.2M NH_4OH pH 10. The solution to be analysed was applied to the column and the column was washed with 25cm^3 of distilled water. Any chromium which adhered to the column was reduced to Cr(III) and eluted using 0.1M H_2SO_4/0.1M ascorbic acid by collecting the first 3cm^3 fraction immediately and then leaving the eluent in contact with the column for 1 hour before the remaining fractions were collected. This allowed time for the reduction of chromium prior to elution. After the hour had elapsed 3cm^3 fractions were collected every minute and the flow rate was retained at 6 drops per 10 seconds, and the fractions were analysed by G.F.A.A.S. using the parameters set out in section 2.1.2.(d).

2.3.5. Establishing Conditions for Elution of Cr(VI) using Ammonium Buffer.

1. (a). Effect of Eluent Concentration.

Using the procedure set out in section 2.3.3. above a 25cm^3 aliquot of 10ppm Cr(VI) in Tris-NaOH pH 13 were applied to the conditioned column, the column was then washed and any Cr(VI) which adhered to the column was then eluted using the following eluents, by collecting 3cm^3 fractions every minute and adding a 2.0cm^3 aliquot of 1.67 x 10^{-3}M DPC to each fraction before being analysed by U.V. Spectroscopy;

(i). 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10. (figure 2.24.(a).)

(ii). 2M $(NH_4)_2SO_4$, 2M NH_4OH pH 10. (figure 2.24.(b).)

(iii). 2M $(NH_4)_2SO_4$, 0.8M NH_4OH pH 10. (figure 2.25.(a).)

(iv). 2M $(NH_4)_2SO_4$, 8.0M NH_4OH pH 10. (figure 2.25.(b).)

It was found that eluent (i) eluted all the chromium from the column in 11 minutes, eluent (ii) took 41 minutes for the elution of the chromium, eluent (iii) did not remove all the chromium from the column after 30 minutes and eluent (iv) took only 10 minutes for the elution of the chromium.

It was also observed that the greater the molarity of the ammonium hydroxide in the eluent, the quicker the Cr(VI) was eluted from the column, but eluent (iv) above required a large volume of concentrated nitric acid to adjust the solution to pH 10. This large volume of nitric acid used would counteract the effect of the ammonia, therefore an optimum eluent was chosen as 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 10. Figures 2.24.(a), 2.24.(b), 2.25(a) and 2.25.(b) represent the elution profiles obtained using each of the above eluents and the data is given in appendix 2.16-2.19 respectively.

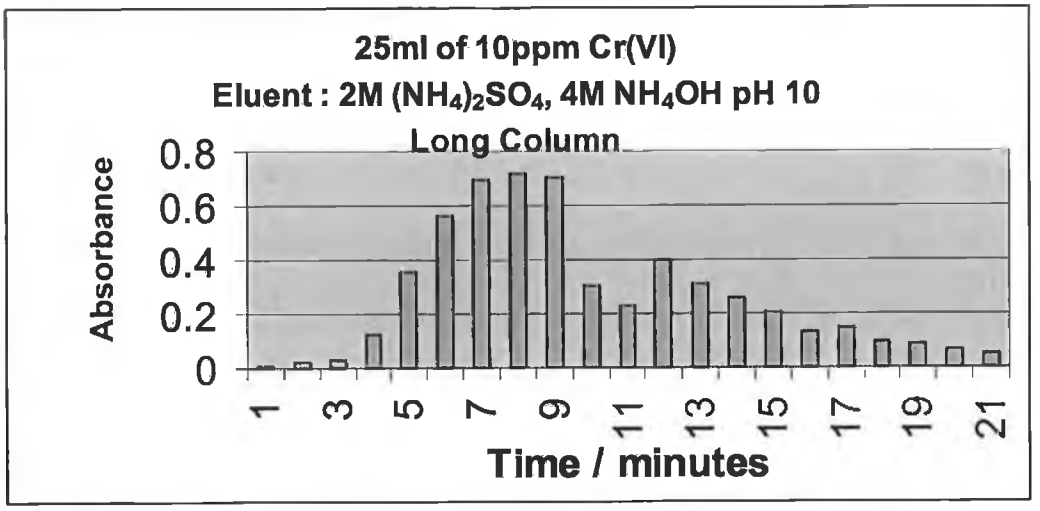


Figure 2.24.(a). Elution profile using a long column and 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 10 as eluent.

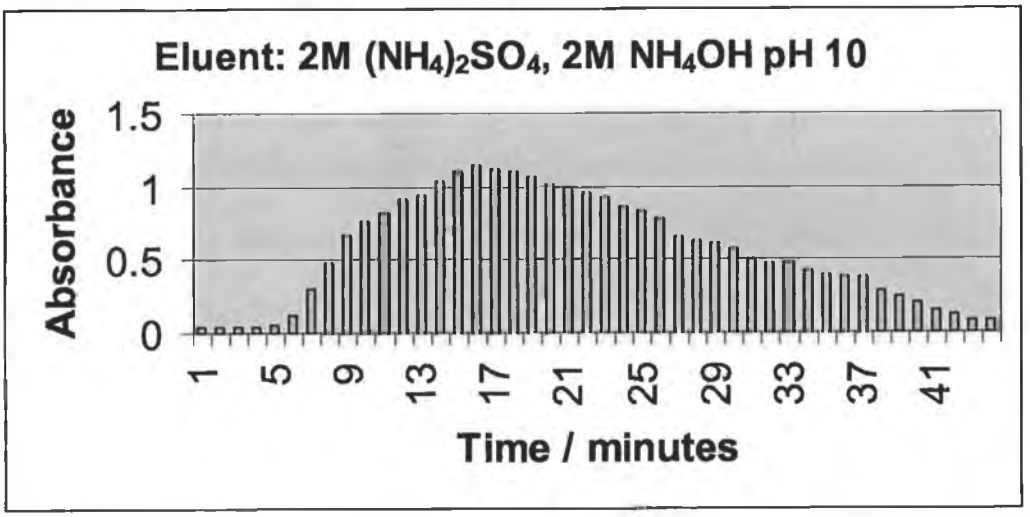


Figure 2.24.(b). Elution profile using a long column and 2M $(NH_4)_2SO_4$, 2M NH_4OH pH 10 as eluent.

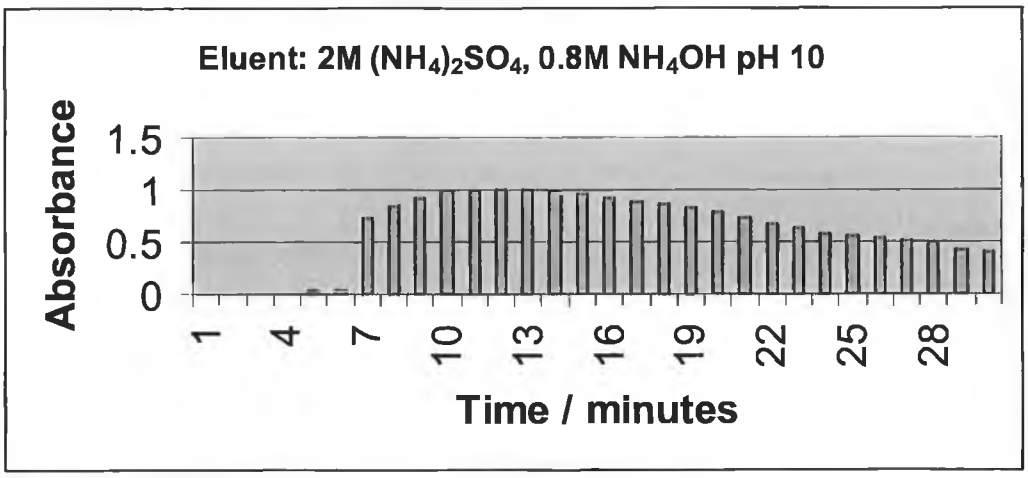


Figure 2.25.(a). Elution profile using a long column and 2M $(NH_4)_2SO_4$, 0.8M NH_4OH pH 10 as eluent.

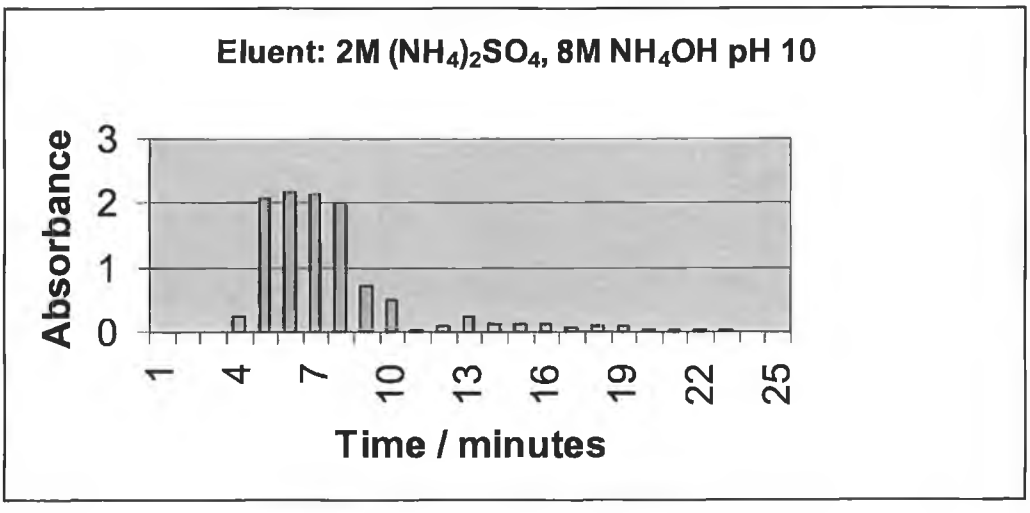


Figure 2.25.(b). *Elution profile using a long column and 2M $(NH_4)_2SO_4$, 8.0M NH_4OH pH 10 as eluent.*

(b). Effect of Eluent pH.

Using the procedure set out in section 2.3.3. a 25cm^3 aliquot of 10ppm Cr(VI) in Tris-NaOH pH 13 were applied to a conditioned column, the column was then washed and any Cr(VI) that adhered to it was then eluted using the following eluents by collecting 3cm^3 fractions every minute and then adding a 2.0cm^3 aliquot of 1.67 x 10^{-3}M DPC to each fraction before being analysed by the U.V. spectrophotometric method in section 2.1.3.(d).

(i). 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 11.9. (figure 2.26.)

(ii). 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 9.0. (figure 2.27.)

(iii). 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10. (figure 2.24.(a).)

It was found that by using eluent (i) the Cr(VI) was eluted in 23 minutes, eluent (ii) gave an elution time of 20 minutes, whereas eluent (iii) gave a much faster elution time of 11 minutes. Therefore an optimum eluent of 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10 was used for the elution of Cr(VI) from the anion exchange in all subsequent experiments where analysis was to be carried out using U.V. spectroscopy. Figure 2.24.(a) represents the elution profile obtained for (iii) above, while 2.26 and 2.27 represent the elution profile obtained for (i) and (ii) above and the data is given in appendix 2.16, 2.20 and 2.21 respectively.

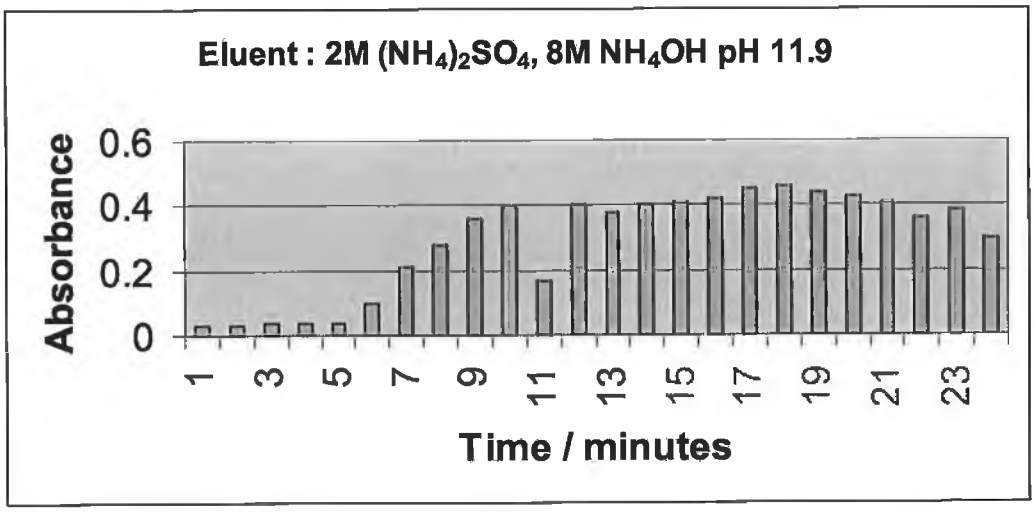


Figure 2.26. Elution profile using a long column and 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 11.9 as eluent.

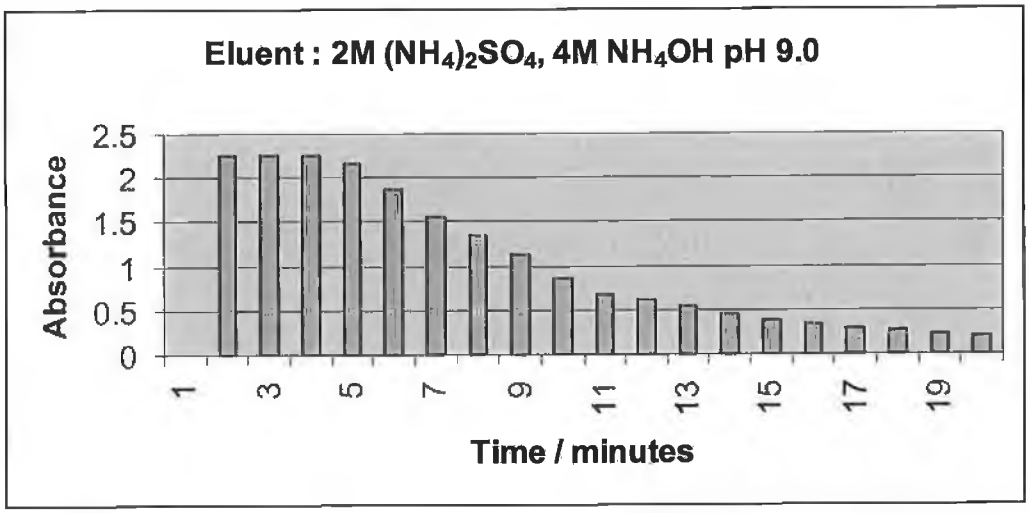


Figure 2.27. Elution profile using a long column and 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 9.0 as eluent.

(c). Effect of Column Length.

Again using the procedure in section 2.3.3. a 25cm^3 aliquot of 10ppm Cr(VI) in Tris-NaOH pH 13 was applied to both the long conditioned column and the short conditioned column (as prepared in section 2.3.1.). Both of these columns were then washed and any Cr(VI) which had adhered to them was then eluted using 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10 again by collecting 3cm^3 fractions manually every minute. A 2.0cm^3 aliquot of 1.67 x 10^{-3}M DPC was then added to each fraction prior to analysis using U.V. spectroscopy. It was found that with the short column the Cr(VI) was eluted in 7 minutes, while it took 11 minutes for the elution of Cr(VI) with the long column, leading to the use of the short column for all further analysis involving ion exchange. Figure 2.24.(a) represents the elution profile for the long column while figure 2.28 represents the elution profile for the short column and the data is given in appendix 2.22.

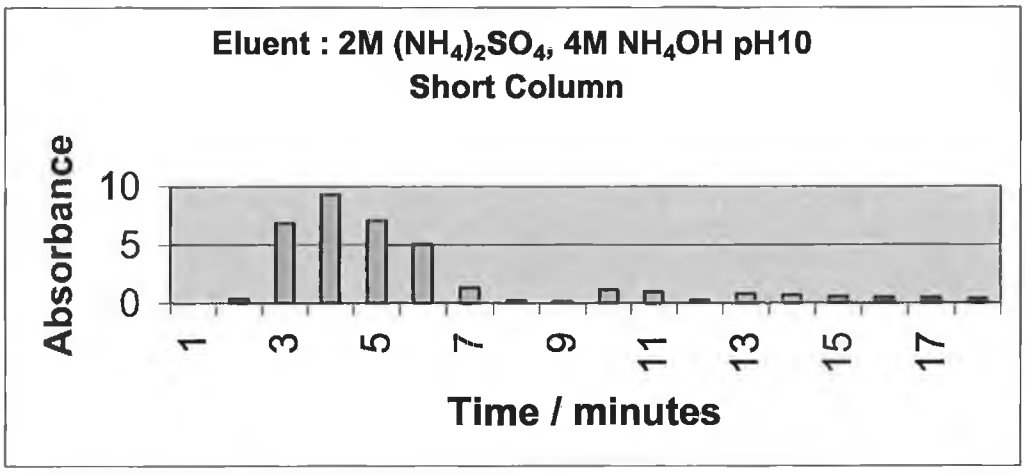


Figure 2.28. Elution profile using a short column and 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 10 as eluent. Absorbance measurements were corrected for dilution.

2. Effect of Analtye Concentration.

Again using the procedure in section 2.3.3. the following aliquots of Cr(VI) concentrations were applied to a conditioned short column, which was then washed and any Cr(VI) which adhered to the column was then eluted using 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10 by collecting $3cm^3$ fractions every minute. A $2.0cm^3$ aliquot of 1.67×10^{-3}M DPC was then added to each of the fractions before analysis was carried out using the U.V. spectrophotometric method in section 2.1.3.(d).

(i). $25cm^3$ of 10ppm Cr(VI) in Tris-NaOH pH 13. (figure 2.28.)

(ii). $25cm^3$ of 1ppm Cr(VI) in Tris-NaOH pH 13. (figure 2.29.)

(iii). $25cm^3$ of 100ppb Cr(VI) in Tris-NaOH pH 13. (figure 2.30.)

(iv). 1 dm^3 of 5ppb Cr(VI) in Tris-NaOH pH 13. (figure 2.31.)

An elution profile was obtained for each of the above Cr(VI) concentrations. Experiment (i) is represented by figure 2.28 above, while (ii), (iii) and (iv) above are represented by figures 2.29-2.31 and the data is given in appendix 2.22-2.25 respectively.

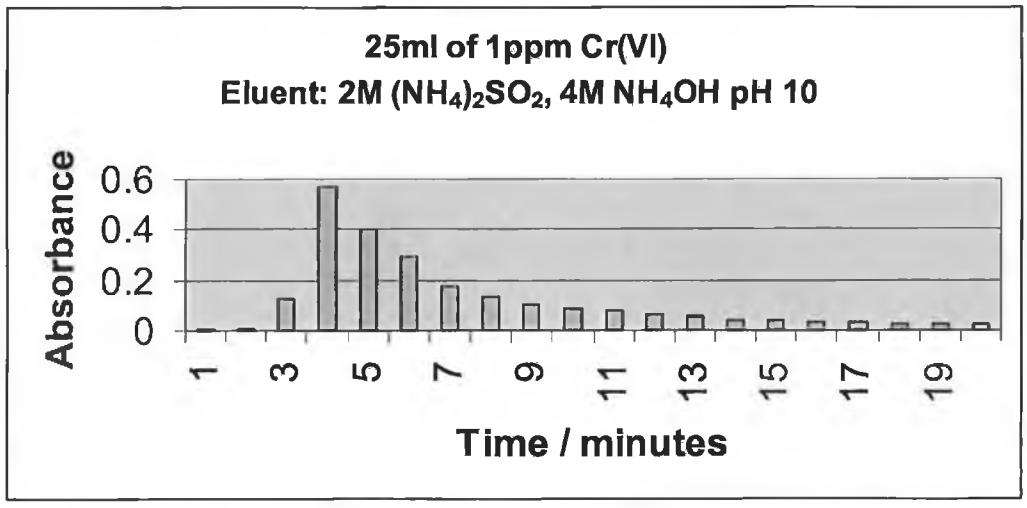


Figure 2.29. Elution profile for $25cm^3$ of 1ppm Cr(VI).

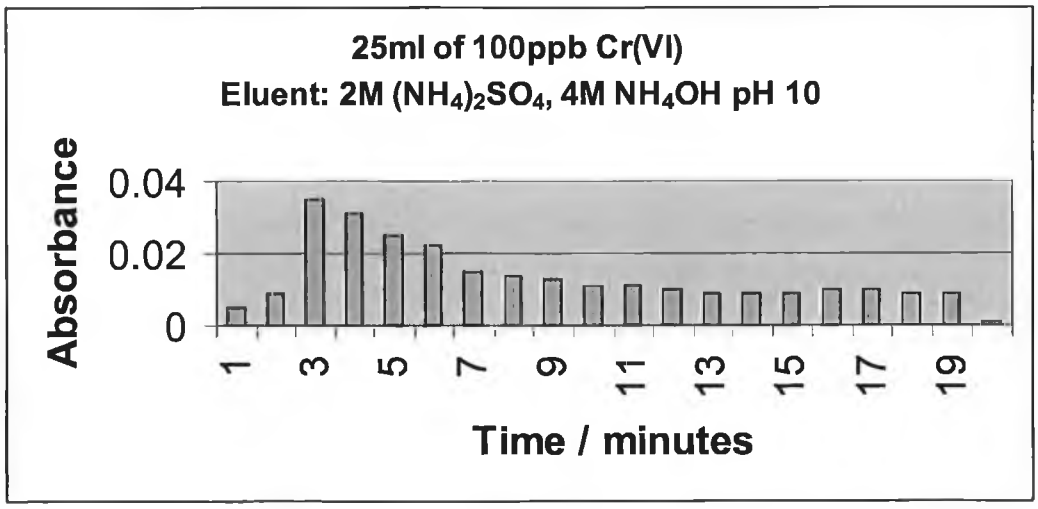


Figure 2.30. Elution profile of 25cm^3 of 100ppb Cr(VI).

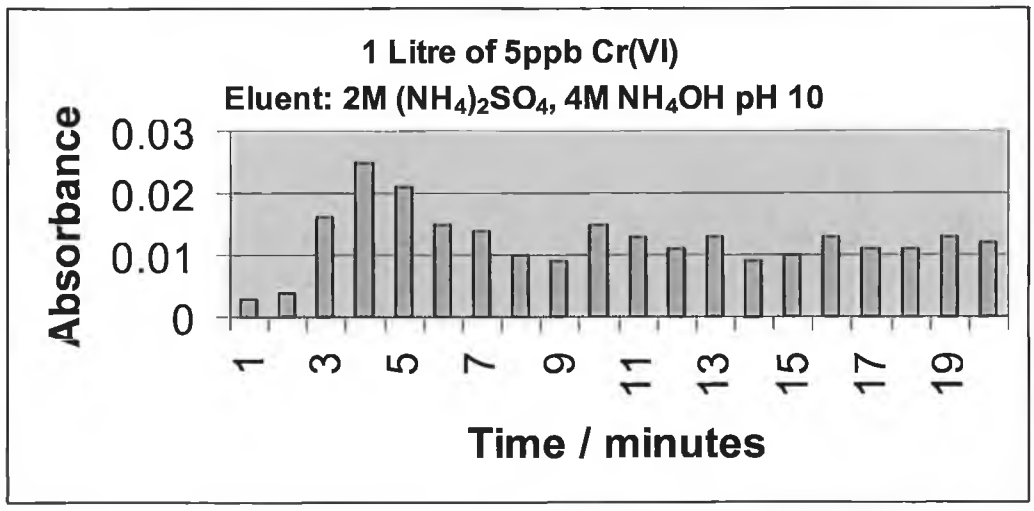


Figure 2.31. Elution profile of 1 dm^3 of 5ppb Cr(VI).

The percentage recovery for (i) and (ii) above were obtained by applying $25cm^3$ of 10ppm and $25cm^3$ of 1ppm Cr(VI) to short conditioned anion exchange columns, which were then washed. Following this any Cr(VI) which adhered to the column was then eluted by collecting a total of $50cm^3$ of 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10 in 1 fraction.

For eluent (i) a $10cm^3$ aliquot of this solution was measured, a $2cm^3$ aliquot of 1.67 x 10^{-3}M DPC was added, the solution was then acidified with concentrated nitric acid and diluted to $100cm^3$ with distilled water. The absorbance measurement of this solution at 540nm was found to be 0.182. A control was also prepared by pipetting a $5cm^3$ aliquot of 10ppm Cr(VI) in Tris-NaOH pH 13 into a $100cm^3$ volumetric flask, a $2cm^3$ aliquot of DPC was added along with $10cm^3$ of 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10. This solution was then acidified using concentrated nitric acid and made up to the mark with distilled water. The absorbance measurement of this solution at 540nm was found to be 0.228 which lead to a percentage recovery of 79.8% being obtained for this solution.

For eluent (ii) a $30cm^3$ aliquot of the fraction was measured, $2cm^3$ of 1.67 x 10^{-3}M DPC was added and the solution was diluted to $50cm^3$ with distilled water. The absorbance measurement of this solution at 540nm was found to be 0.088. A control was then prepared by pipetting a $15cm^3$ aliquot of 1ppm Cr(VI) in Tris-NaOH pH 13 into a $50cm^3$ volumetric flask, $2cm^3$ of DPC was then added along with $30cm^3$ of 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10 and the solution was acidified and made up to the mark with distilled water. The absorbance measurement of this solution was obtained at 540nm and found to be 0.121 giving a percentage recovery of 72.7%.

For eluent (iii) above the percentage recovery was obtained by applying $25cm^3$ of 100ppb Cr(VI) to a conditioned anion exchange column, which was then washed.

Any Cr(VI) which adhered to the column was then eluted by collecting a total of 25cm^3 of 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10 in 1 fraction. 20cm^3 of this solution was then pipetted into a 25cm^3 volumetric flask, a 2cm^3 aliquot of 1.67 x 10^{-3}M DPC was added and the solution was made up to the mark with distilled water. The absorbance measurement of this solution at 540nm was found to be 0.055. A control was then prepared by pipetting a 2cm^3 aliquot of 1ppm Cr(VI) in Tris-NaOH pH 13 into a 25cm^3 volumetric flask, 2cm^3 of 1.67 x 10^{-3}M DPC were then added along with 20cm^3 of 2M $(NH_4)_2SO_2$, 4M NH_4OH pH 10 and the solution was acidified and made up to the mark with distilled water. The absorbance measurement of this solution was obtained at 540nm and found to be 0.081 giving a percentage recovery of 67.9%.

Table 2.13. Percentage recovery figures for various Cr(VI) standards.

Conc. Of Cr(VI) / ppm	% Recovery.
10	79.8
1	72.7
0.1	67.9

From the percentage recovery figures listed in Table 2.13 it can be seen that as the concentration of Cr(VI) decreased so too did the percentage recovery.

2.3.6. Preconcentration of Cr(VI) from Yeast Extracts.

The extraction of 1g of enriched yeast and 1g of non-enriched yeast were carried out in the same way as outlined in section 2.2.2.(c). Extraction was carried out under nitrogen and the filtered extract was applied directly to the column without further dilution. For solution (iii) below 0.0103g of $Cr(III)Cl_3$ was added to 1g of non-enriched yeast and the same extraction procedure as for the chromium enriched and the non-enriched yeast above was carried out. These extraction procedures produced the following;

(i). $42cm^3$ of Cr extract from chromium enriched yeast.

(ii). $43cm^3$ of Cr extract from non-enriched yeast.

(iii). $45cm^3$ of Cr extract from non-enriched yeast containing 20ppm Cr(III).

The preconcentration and elution procedure described above was carried out on these solutions by applying them to a conditioned column, which was then washed. Any Cr(VI) which had adhered to the column was then eluted with 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 10 by collecting $3cm^3$ fractions every minute. A $2cm^3$ aliquot of 0.1M H_2SO_4 was then added to each fraction, to give a pH of 2, along with 400µL of 1.67×10^{-3}M DPC and each fraction was analysed using the spectrophotometric procedure described in section 2.1.3.(d).

Figure 2.32-2.34 represent the elution profiles obtained for these extractions and the data is given in appendix 2.26-2.28 respectively. Sample (i) above did produce a typical elution profile but the absorbance measurements obtained were low. It is possible that air oxidation of Cr(III) may occur during the ion exchange process but this did not occur with sample(iii). Exclusion of air during the ion exchange process would be necessary to completely eliminate this possibility.

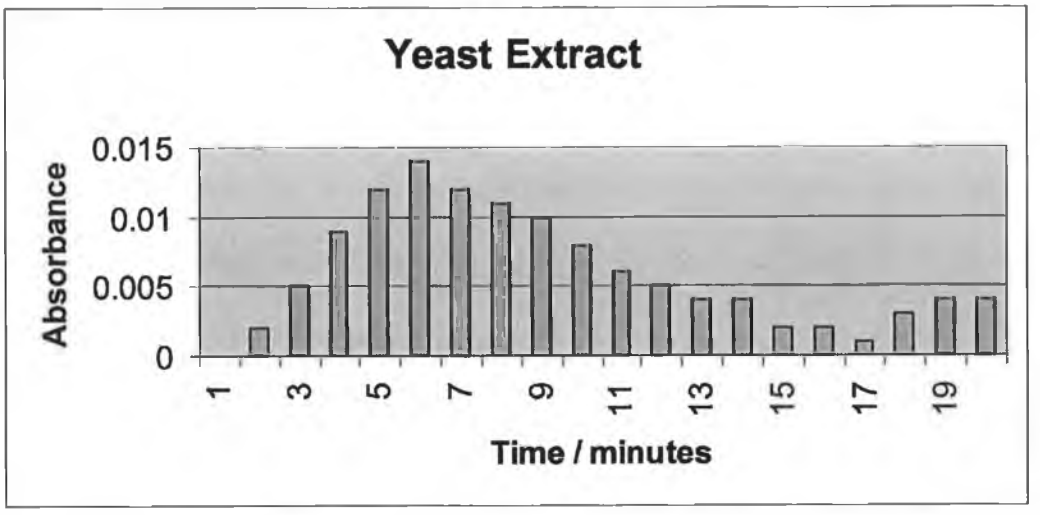


Figure 2.32. Elution profile for the extracted solution from chromium enriched yeast.

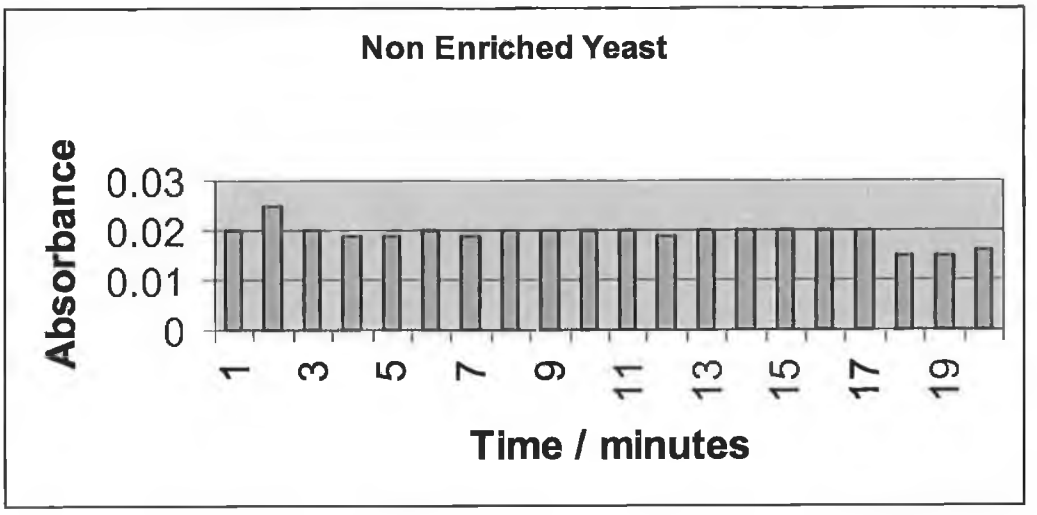


Figure 2.33. Elution profile for the extracted solution from non enriched yeast.

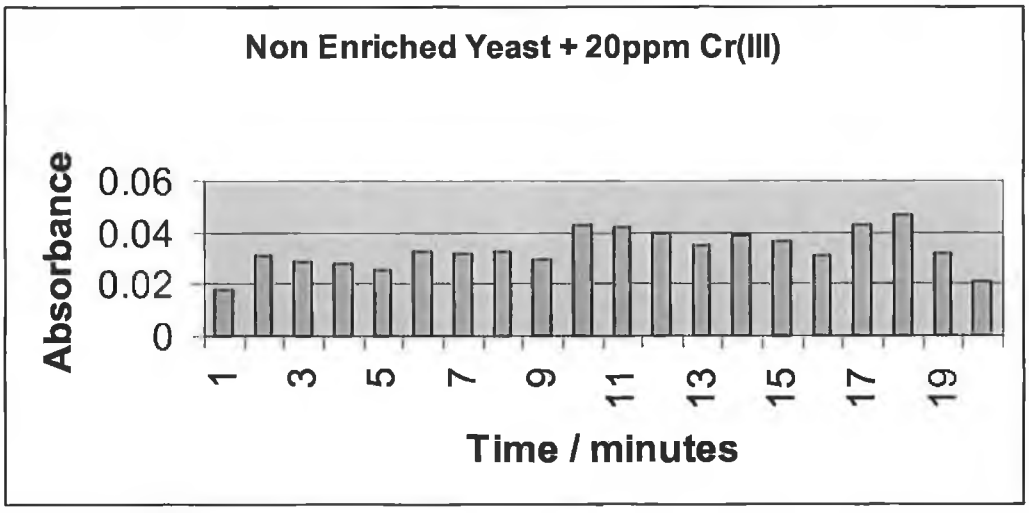


Figure 2.34. Elution profile of the extracted solution from non enriched yeast + 20ppm Cr(III).

2.3.7. Establishing Conditions for the Elution of Cr(VI) using H_2SO_4 and Ascorbic Acid.

The principle of this method of preconcentration differs from that previously used. It was found that G.F.A.A. spectroscopy would not tolerate the high ionic strength of the $(NH_4)_2SO_4$, NH_4OH buffer used in the previous section. Therefore Cr(VI) was eluted from the column by reduction with acidic ascorbic acid. The eluted fractions were readily analysed by G.F.A.A. spectroscopy, which is more sensitive than the Cr-DPC spectrophotometric method.

1. Ascorbic Acid Reduction Procedure.

(i). $25cm^3$ of 1ppm Cr(VI) in 0.2M Tris-NaOH pH 13 was applied to the anion exchange column, which had been conditioned with $100cm^3$ of 0.2M NH_4OH pH 10 and then washed with $25cm^3$ of distilled water. Any chromium which adhered to the column was then reduced and eluted by collecting $3cm^3$ fractions every minute, using 0.1M H_2SO_4 / 0.1M ascorbic acid as eluent and the absorbance measurement of each

fraction was obtained using the G.F.A.A. spectroscopy method outlined in section 2.1.2.(d). Figure 2.35 represents the elution profile that was obtained and the data is given in appendix 2.29. It was found that no chromium was detected in the first 3 fractions and then there was a sudden rise in absorbance measurements.

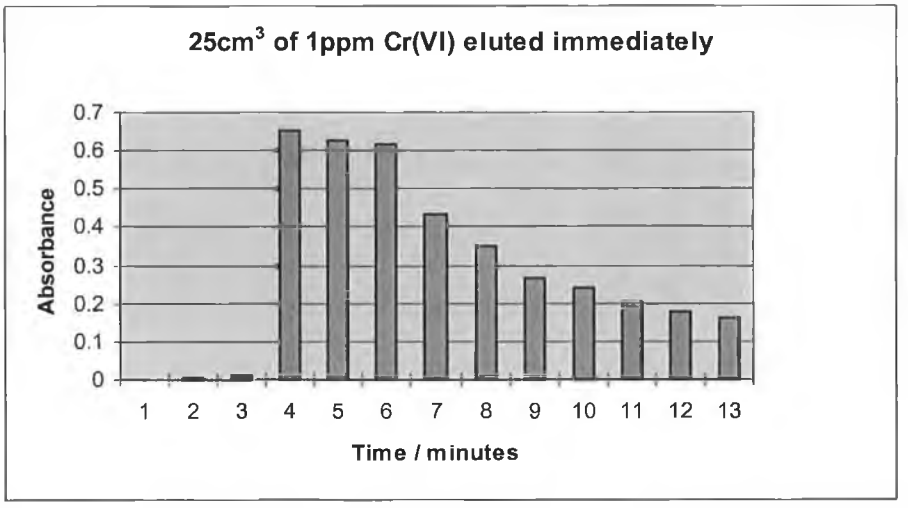


Figure 2.35. Elution profile obtained for 25cm^3 of 1ppm Cr(VI) eluted immediately.

(ii). Again 25cm^3 of 1ppm Cr(VI) in 0.2M Tris-NaOH pH 13 was applied to an anion exchange column in the same way as the previous experiment. The first 3cm^3 fraction was collected immediately and the eluent was then left in contact with the anion exchange column for 1 hour before the remaining fractions were collected, again a 3cm^3 fraction every minute. Each of these fractions were again analysed using G.F.A.A. spectroscopy and the elution profile obtained is in figure 2.36 and the data is given in appendix 2.30. A more ideal elution profile was obtained for this experiment than was obtained for the previous experiment since it was found that the majority of the chromium was reduced and eluted from the column between 7 and 12 minutes. This was so because by leaving the eluent in contact with the column for 1 hour prior to elution of the Cr(VI) allowed time for any chromium on the column to be reduced to Cr(III) before elution took place.

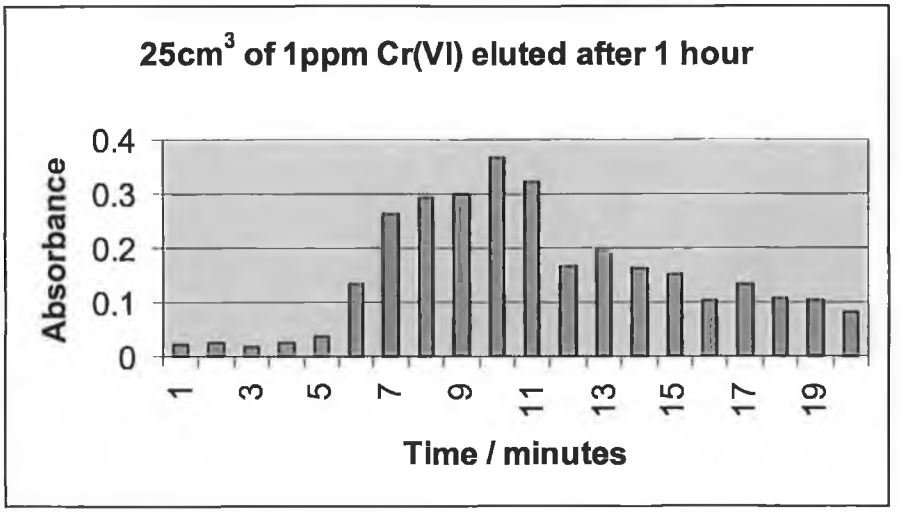


Figure 2.36. Elution profile obtained for 25cm^3 of 1ppm Cr(VI) eluted after 1 hour.

2. Elution of Cr(VI) Standards.

The following Cr(VI) standards were preconcentrated and eluted from an anion exchange column by applying them to a conditioned column and then reducing and eluting the Cr(VI) using 0.1M H_2SO_4 / 0.1M ascorbic acid as eluent and the above procedure, where elution was carried out after the eluent was left in contact with the column for 1 hour, by collecting the first 3cm^3 fraction immediately and the remaining 3cm^3 fractions after 1 hour;

(i). 25cm^3 of 1ppm Cr(VI) in Tris-NaOH pH 13.

(ii). 25cm^3 of 100ppb Cr(VI) in Tris-NaOH pH 13.

(iii). 100cm^3 of 10ppb Cr(VI) in Tris-NaOH pH 13.

(iv). 1 dm^3 of 1ppb Cr(VI) in Tris-NaOH pH 13.

All the fractions from the above procedures were analysed using the G.F.A.A. spectroscopic method as outlined in section 2.1.2.(d). and an elution profile was

obtained for each of the standards. The elution profile for (i) above is shown in figure 2.36 while the elution profile for (ii), (iii) and (iv) are shown in figures 2.37-2.39 and the data is given in appendix 2.31-2.33 respectively. It was found that using this preconcentation and reduction procedure as low as 1 ppb Cr(VI) can be detected.

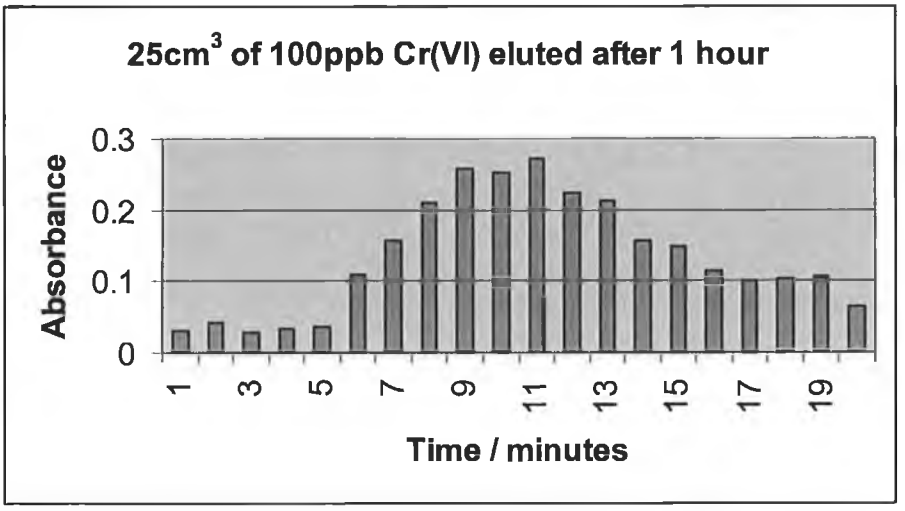


Figure 2.37. Elution profile for 25cm^3 of 100ppb Cr(VI) eluted after 1 hour.

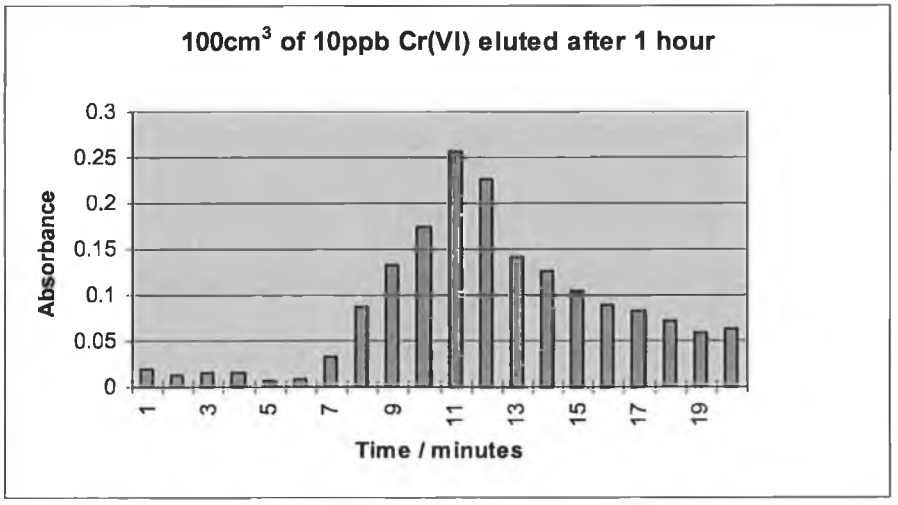


Figure 2.38. Elution profile for 100cm^3 of 10ppb Cr(VI) eluted after 1 hour.

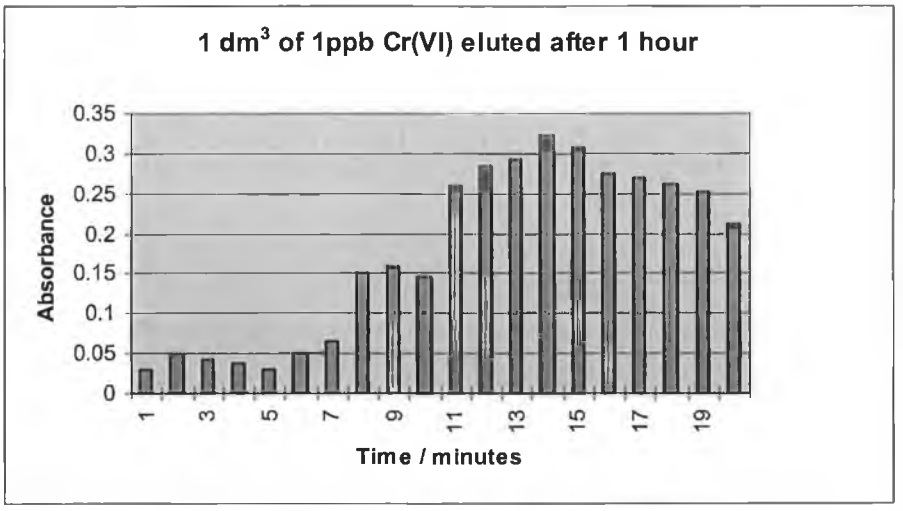


Figure 2.39. Elution profile for 1 dm^3 of 1ppb Cr(VI) eluted after 1 hour.

2.3.8. Preconcentration of Cr(VI) from Yeast Extract.

The extraction procedure in section 2.2.2.(c) was carried out on 1.0002g of the chromium enriched yeast and 1.0005g of the non enriched yeast but the final filtrate was not made up to 100cm^3, i.e. the extracted solution was left undiluted. This procedure produced 45cm^3 of extracted solution for both extractions. The above preconcentration, reduction and elution procedure was carried out on the 45cm^3 of the extracted solution of both the enriched yeast and the non-enriched yeast. Absorbance measurements were obtained using G.F.A.A. spectroscopy. A large puff of smoke was obtained during the ashing procedure which leads to elevated readings. Even in the case of the non-enriched yeast which contains no added chromium the elution profiles indicate the presence of Cr(VI). No confidence can be placed in the results which are given below. Figure 2.40 shows the elution profile obtained for the extracted solution of the chromium enriched yeast and the data is given in appendix 2.34, while figure 2.41 shows the elution profile for the extracted solution of the non enriched yeast and the data is given in appendix 2.35.

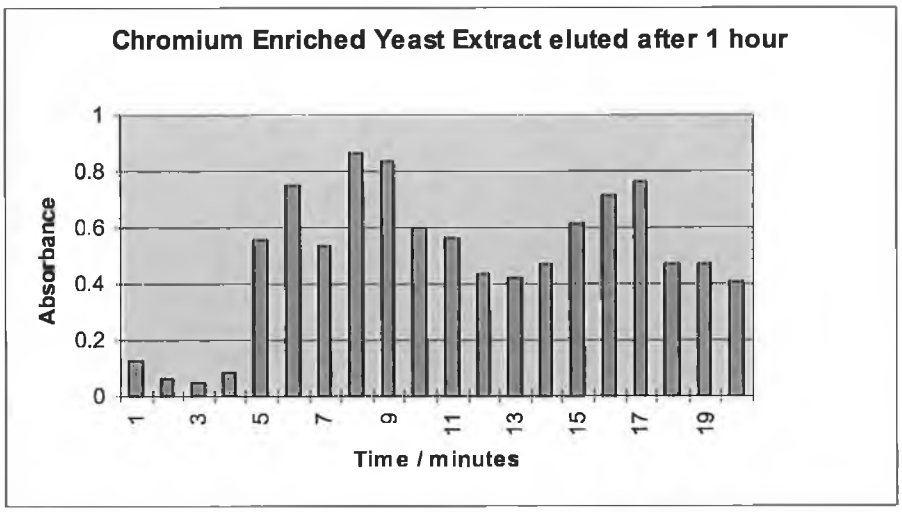


Figure 2.40. Elution profile obtained for the extract of the chromium enriched yeast eluted after 1 hour.

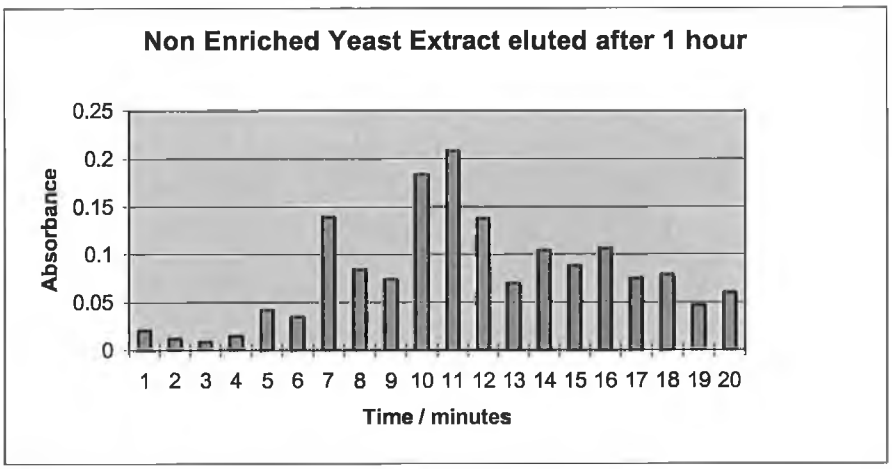


Figure 2.41. Elution profile obtained of the extract from the non enriched yeast eluted after 1 hour.

2.3.9. Separation of Cr(VI) and Cr(III) by Ion Exchange.

In this section the separation of Cr(VI) from an excess of Cr(III) by ion exchange was investigated. This is the situation encountered in the yeast extract. Under alkaline conditions Cr(VI) has a negative charge and is retained on an anion exchange resin. Under alkaline conditions Cr(III) forms negatively charged complexes such as $Cr(OH)_6^{3-}$ (aq) which will be retained on an ion exchange resin. There may also be complexing by the Tris-NaOH buffer. Theoretically Cr(III) complexation would be reversed in acid solution allowing the retained Cr(III) to be removed from the column using acid solution. Cr(VI) is anionic at all pH values and would only be removed by a high ionic strength buffer such as 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 10 or by a reducing buffer such as ascorbic acid, H_2SO_4.

(a). Separation of 50ppm Cr(III) and 100ppb Cr(VI) in Tris-NaOH pH 13.

A control was prepared by pipetting a $5cm^3$ aliquot of 50ppm Cr(III) and 100ppb Cr(VI) in 0.2M Tris-NaOH pH 13 solution into a $10cm^3$ volumetric flask and making up to the mark with 0.2M Tris-NaOH pH 13, and the absorbance measurement was obtained using flame A.A. and found to be 0.0994.

An anion exchange column as prepared in section 2.3.1. was conditioned with $100cm^3$ of 0.2M NH_4OH pH 10. A $25cm^3$ aliquot of 50ppm Cr(III) and 100ppb Cr(VI) in Tris-NaOH pH 13 was applied to the column at a rate of 6 drops per 10 seconds, and the solution which passed through the column was collected. The column was then washed with distilled water, which was collected along with what had passed through the column until a total of 50 cm^3 had been collected. The absorbance measurement of this solution was again obtained using flame A.A. and was found to be 0.0757. By

comparing this absorbance measurement with that of the control the percentage of the chromium(III) which passed through the column was 76.2%.

In alkaline solution Cr(III) was retained on the column as anionic hydroxy complexes. Acidification should destroy these complexes. The Cr(III) was eluted from the column using 0.1M H_2SO_4. The acidic solution was passed through the column for 1 minute and a 3 cm^3 fraction was collected. The acid was allowed to stay in contact with the column for 1 hour and then 3cm^3 fractions were collected every minute for 9 minutes. The absorbance measurement of each fraction was measured using flame A.A. It was found that any Cr(III) on the column was eluted within 9 minutes. The elution profile obtained here is shown in figure 2.42 and the data is given in appendix 2.36.

Cr(VI) is anionic at all pH values and would still be retained on the column. The Cr(VI) on the column was eluted using 2M $(NH_4)_2SO_4$ / 4M NH_4OH pH 10 by collecting 3cm^3 fractions every minute. A 2cm^3 aliquot of 1.67 x 10^{-3}M DPC was then added to each fraction and the absorbance measurements were obtained at 540nm as in section 2.1.3.(d). A typical elution profile was obtained and it was found that the Cr(VI) was eluted from the column in 7 minutes. The elution profile is shown in figure 2.43 and the data is given in appendix 2.37.

From the above experiment it has been shown how a trace concentration of Cr(VI) (100ppb) can be separated from a large excess (50 ppm) of Cr(III) and detected spectrophotometrically using DPC.

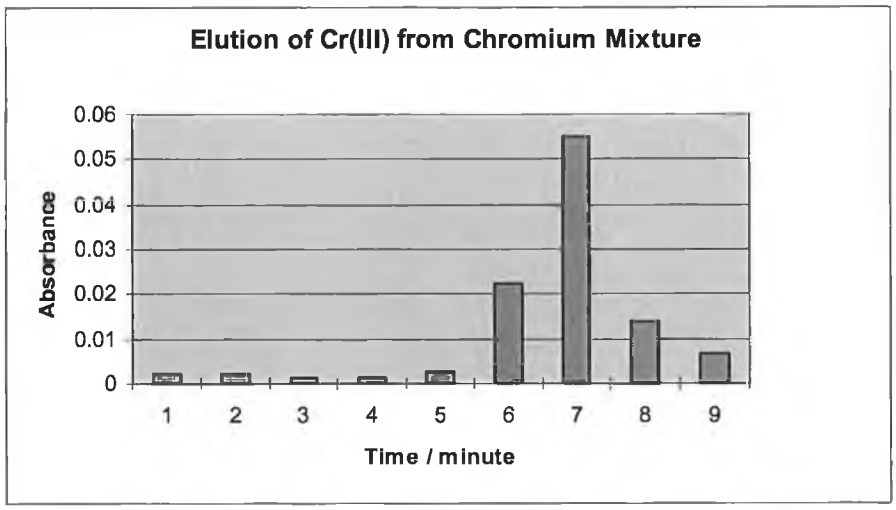


Figure 2.42. Elution profile obtained for Cr(III) from chromium mixture.

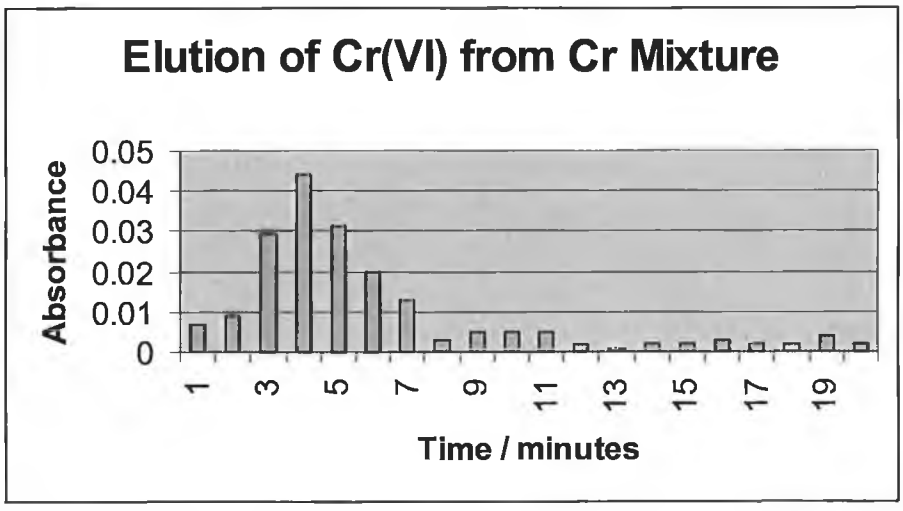


Figure 2.43. Elution profile obtained for Cr(VI) from chromium mixture.

In order to prevent air oxidation of Cr(III) during the extraction of the chromium yeast the procedure was carried out under nitrogen. It was necessary to demonstrate that no air oxidation occurs during the process of extraction followed by ion exchange before the procedure described in section (a) could be applied to the yeast extract. Therefore the extraction process was simulated using a 50ppm Cr(III) solution in tris NaOH buffer at pH 13. A 45 cm^3 aliquot of 0.2M tris NaOH pH 13 was deaerated for 15 minutes and 5 cm^3 of 1000 ppm Cr(III) was added and deaerated for a further 5 minutes. The mixture was left stirring overnight. Next day this solution was centrifuged and filtered as described previously in section 2.2.2.(c). A 25cm^3 aliquot of this extract was then applied to an anion exchange column as in the previous experiment and eluted with distilled water until a total of 50cm^3 had been collected. The absorbance of the solution was found to be 0.0738±0.000499 using flame A.A.

A control was prepared by pipetting 5cm^3 of 50ppm Cr(III) in 0.2M Tris-NaOH pH 13 into a 10cm^3 volumetric flask and making up to the mark with deaerated distilled water and the absorbance measurement of this was found to be 0.0929 ±0.000916 by flame A.A. The percentage Cr(III) which passed through the column was found to be 79.0%.

As in the previous experiment a solution of 0.1M H_2SO_4 was passed through the column to elute any Cr(III) which was present by collecting 3cm^3 fractions every minute. After the first fraction was collected the eluent was left in contact with the column for 1 hour after which the remaining fractions were collected and analysed using flame A.A. It was found that the Cr(III) was eluted from the column in 9 minutes. The elution profile is shown in figure 2.44 and the data is given in appendix 2.38.

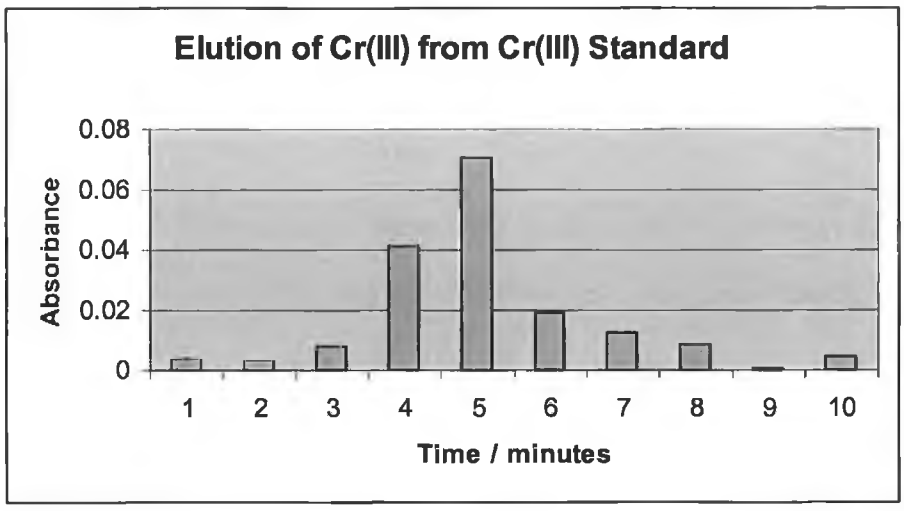


Figure 2.44. Elution profile obtained for Cr(III) from Cr(III) standard.

Following this any Cr(VI) that may have adhered to the column was eluted from the column using 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 10 by collecting $3cm^3$ fractions every minute and analysis was carried out by adding a $2.0cm^3$ aliquot of 1 x 10^{-3}M DPC to each fraction and obtaining the absorbance measurement at 540nm. Since no colour change was observed after the addition of DPC to the fractions the experiment indicates that no Cr(VI) was present in the fractions and indicates that Cr(III) was not oxidized to Cr(VI) while on the column or during the elution process. The elution profile for this experiment is shown in figure 2.45 and the data is given is appendix 2.39. There is negligible absorbance in the 3-6 minute fractions where Cr(VI) would be eluted.

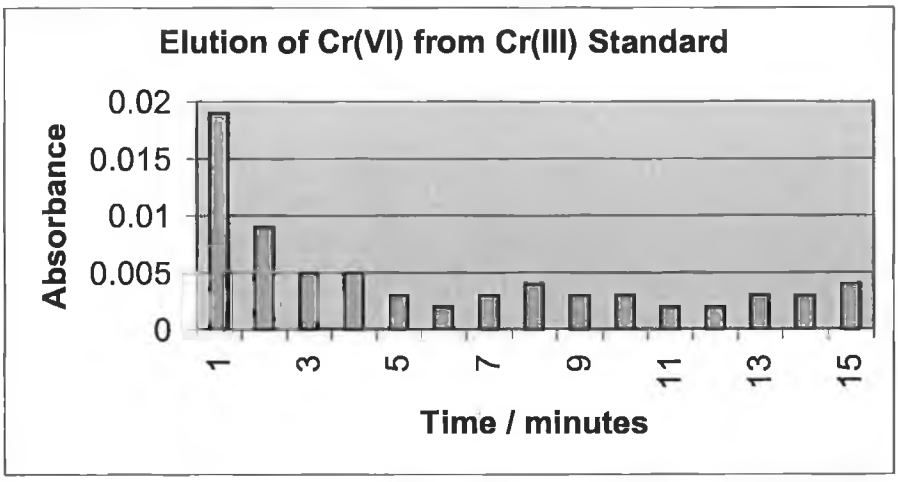


Figure 2.45. Elution profile obtained for Cr(VI) from Cr(III) standard.

(c). Separation of Cr(III) and Cr(VI) from a Chromium Enriched Yeast Extract.

The extraction procedure as in section 2.2.2.(c) was carried out on 0.9992g of the chromium enriched yeast. A 25cm^3 of this extract was then applied to an anion exchange column, again as in the previous experiment, and the absorbance measurement of the solution which passed through the column along with the rinsings was found to be 0.0589 using flame AA.

A control was prepared by pipetting 5cm^3 of the chromium enriched yeast extract into a 10cm^3 volumetric flask and making up to the mark with deaerated distilled water. The absorbance measurement of this solution was found to be 0.0755 using flame AA. The percentage Cr(III) which passed through the column was found to be 78%.

As in the previous separation experiment the 0.1M H_2SO_4 was passed through the column to elute any Cr(III) present by collecting 3cm^3 fractions every minute. The eluent was allowed to remain in contact with the column for 1 hour after the first fraction was collected, after which the remaining fractions could be collected at 1

minute intervals. The fractions were analysed using flame A.A. Again it was found that the Cr(III) was eluted from the column in 9 minutes. The elution profile obtained is shown in figure 2.46 and the data is given in appendix 2.40.

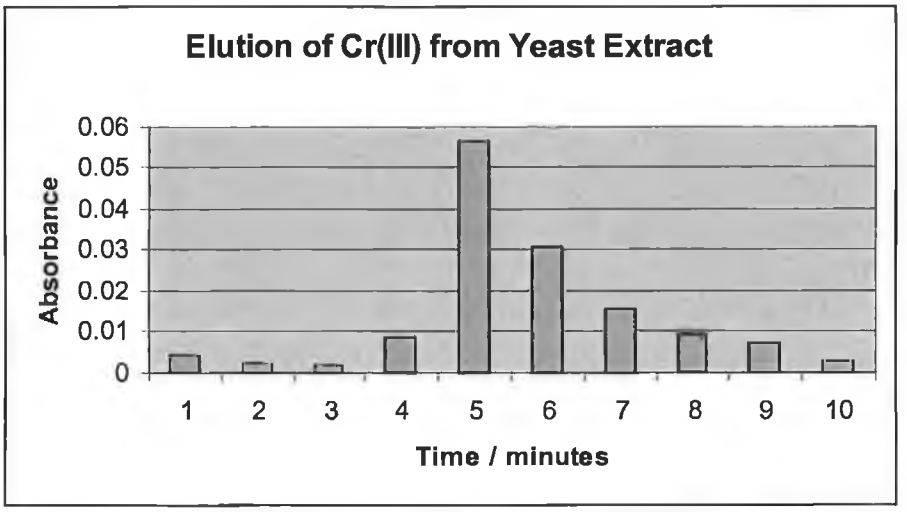


Figure 2.46. Elution profile obtained for Cr(III) from the chromium enriched yeast extract.

Following this any Cr(VI) which may have adhered to the column was eluted from the column with 2M $(NH_4)_2SO_4$, 4M NH_4OH pH 10 again by collecting $3cm^3$ fractions every minute and the analysis was carried out by adding $2cm^3$ of 1.67×10^{-3}M DPC to each fraction and the absorbance measurement was obtained at 540nm. The elution profile is shown in figure 2.47 and the data is given in appendix 2.41. No colour change was observed here after the addition of the DPC to the fractions and no evidence for the presence of Cr(VI) in the extracts was observed. The result suggests that that there is no Cr(VI) in the yeast extract.

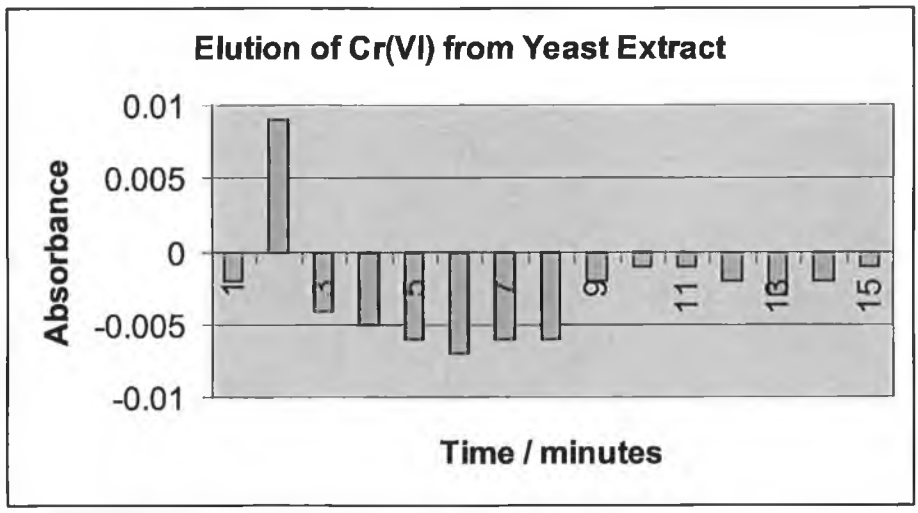


Figure 2.47. Elution profile obtained for Cr(VI) from the chromium enriched yeast extract.

This result indicates that if the Cr(III) is separated from any Cr(VI) in the yeast extract prior to elution of any adsorbed Cr(VI), air oxidation of Cr(III) cannot occur during the latter process. This suggests that the elution profile obtained in figure 2.32 (p 168) may have been due to air oxidation.

2.4. PRECONCENTRATION OF Cr(VI) BY H.P.L.C..

2.4.1. Reagents and Solutions.

1. Acetonitrile, CH_3CN, F.W.=41.05g mol^{-1}, BDH Chemicals Ltd., #29220.
2. Sulphuric Acid, H_2SO_4, F.W.=98.07g mol^{-1}, S.G.=1.84, Romil Ltd., Code A-9691.
3. 1,5-Diphenylcarbazide, $C_{13}H_{14}NO_4$, F.W.=242.3g mol^{-1}, Aldrich Chemical Company Inc., #25922-5.
4. Potassium Dichromate, $K_2Cr_2O_7$, F.W.=294.19g mol^{-1}, Wardle Chemical Company Ltd., #PO4868.
5. Chromium Enriched Yeast, Alltech Ireland,Dunboyne, Co. Meath.

Preparation of Solutions.

1. Mobile Phase. 6 x 10^{-3}M H_2SO_4 in 20% Acetonitrile.

   As in section 2.1.4.(b).

2. 2 x 10^{-3}M DPC in 10% Acetonitrile, 0.048M H_2SO_4.

   As in section 2.1.4.(b).

3. 1ppm Cr(VI).

   As in section 2.3.1.(c).

2.4.2. Instrumentation.

UV Spectrophotometric Detector SPD-6A,Shimadzu.

Liquid chromatography LC-6A, Shimadzu.

3390A Integrator – Hewlett Packard Model 1.

2.4.3. Analysis of Chromium Enriched Yeast Extract.

The extraction procedure as in section 2.2.2.(c). (i.e. under nitrogen) was carried out on 1.0021g of chromium enriched yeast, but before the final filtration step was carried out, the extract was adjusted to pH 2 using 5M H_2SO_4. To $20cm^3$ of this extracted solution, without the final dilution (i.e. the filtrate was not made up to $100cm^3$ with deaerated 0.2M Tris-NaOH pH13), a $2.5cm^3$ aliquot of 2.x 10^{-3}M DPC was added and the preconcentration and elution as outlined in section 2.1.4.(d). was carried out.

It was found that no colour change was observed after the addition of the DPC solution and no Cr(VI) peak appeared after elution indicating that no Cr(VI) was present in the chromium enriched yeast extract. The chromatogram for the extract is given in figure 2.48.(a).

2.4.4. Reduction of Trace Cr(VI) by Chromium Enriched Yeast Extract.

To a $20cm^3$ aliquot of the extracted solution from the chromium enriched yeast above the following Cr(VI) concentrations were added, followed by a $2.5cm^3$ aliquot of 2 x 10^{-3}M DPC and the preconcentration and elution procedure from section 2.1.4.(d). was carried out. By addition of the aliquots of Cr(VI) listed below to $20cm^3$ of the extracted solution the following concentrations of Cr(VI) were produced; (a) 13ppb Cr(VI), (b) 44ppb Cr(VI) and (c) 88ppb Cr(VI).

(a). 300µL of 1ppm Cr(VI).

(b). 100µl of 10ppm Cr(VI).

(c). 200µl of 10ppm Cr(VI).

It was found that for (a) above a very small Cr(VI) peak of area 1612 was observed at 8.06 minutes after elution and on comparing this peak area with that of the 15ppb Cr(VI) from the calibration curve in section 1.2.4.(d) it implies that the extracted solution from the chromium enriched yeast reduced the majority of the 13ppb Cr(VI) that was added. For (b) a small peak at 8.29 minutes was observed of area 6879 and for (c) a larger peak of area 307170 was observed at 8.24 minutes, and

again on comparing these peak areas with those of the Cr(VI) standards in the calibration curve it can be seen that the peak areas obtained for both of these peaks are greatly reduced compared to what would be expected from a 44ppb and an 88ppb Cr(VI) solution, implying again that the chromium enriched yeast extract can reduce Cr(VI). From these experiments it appears that the chromium enriched yeast is capable of rapidly reducing Cr(VI) below 13ppb. Figure 2.48. shows the chromatograms obtained after the preconcentration and elution of the above solutions.

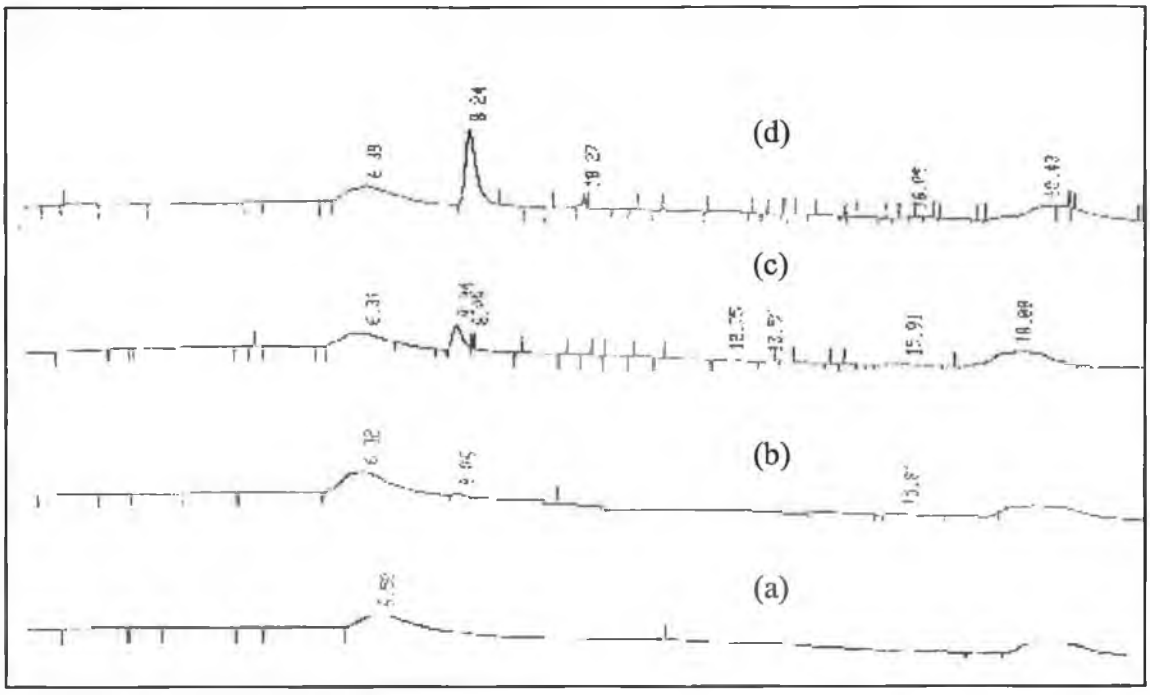


Figure 2.48. Chromatograms obtained for (a) chromium enriched yeast extract, (b), (c) and (d) chromium enriched extract plus 13ppb, 44ppb and 88ppb Cr(VI) respectively.

The solutions were not tested over time, to see if further reduction of Cr(VI) occurred.

2.5. Reduction Capacity of Yeast.

2.5.1. Reduction of Cr(VI) by Ascorbic Acid.

2.5.1.(a). Introduction.

According to Connett (1985) the rate determining step in the reaction of ascorbic acid and Cr(VI) is the formation of a Cr(VI) ester, which rapidly decomposes into the products, Cr(VI) and dehydroascorbic acid. The rate of the reaction is given by

$$\text{Rate} = k[\text{Ascorbic Acid}]\,[\text{Cr(VI)}] \qquad (1)$$

where k = second order rate constant

In order to determine k the reaction was carried out in excess ascorbic acid. Under these conditions the rate is given by

$$\text{Rate} = k_{obs}\,[\text{Cr(VI)}] \qquad (2)$$

where k_{obs} = the pseudo-first-order-rate constant

By combining equations (1) and (2) it is clear that

$$k_{obs} = k[\text{Ascorbic Acid}] \qquad (3)$$

By plotting k_{obs} against [Ascorbic Acid] the second order rate constant for the reaction may be determined.

Determination of First Order Rate Constants.

For a first order reaction the concentration (C) of the reactant decreases with time

$$C = C_o e^{-k_{obs}\,t} \qquad (4)$$

Assuming that the concentration of Cr(VI) is proportional to the absorbance at 375nm the absorbance at this wavelength may be used to follow the decrease in Cr(VI) concentration.

$$A = A_o e^{-k_{obs}\,t} \qquad (5)$$

A plot of A verses time/seconds will be exponential. The data may be fitted to equation 5 using non linear least squares and the values for A_o and k_{obs} can be obtained. In this section the first order rate constant was determined at 298K, at various ascorbic acid concentrations.

2.5.1.(b). Reagents and Solutions.

Reagents.

1. Trizma (Tris[hydroxymethyl]aminomethane) hydrochloride, $C_4H_{11}NO_3.HCl$, F.W.=157.6g mol^{-1}, Sigma, #T3254.
2. Ascorbic Acid, $C_6H_8O_6$, F.W. = 176.13 g mol^{-1}, BDH Biochemicals, #44006.
3. Potassium Dichromate, $K_2Cr_2O_7$, F.W. = 294.49g mol^{-1}, Wardle Chemical Company Ltd., #PO4868.
4. Sulphuric Acid, H_2SO_4, S.G. = 1.84, F.W. = 98.07g mol^{-1}, Romil Ltd., Code A-9691.
5. Sodium Hydroxide, NaOH, F.W. = 40.00g mol^{-1}, Wardle Chemicals Ltd., #SO5652.
6. Tris(hydroxymethyl)aminomethane, $C_4H_{10}NO_3$, F.W.= 121.12g mol^{-1}, Riedel-de Haen, #33742.

Preparation of Solutions.

1. 1Molar Tris-HCl pH 7.4.

157.6001g of Tris(hydroxymethyl)-aminomethane were dissolved in 1 dm^3 of distilled water and the pH was adjusted to 7.4 using 5M NaOH.

2. *Ascorbic Acid Standards.*

Each of the quantities of ascorbic acid listed in Table 2.14 were dissolved in $90cm^3$ of 1M Tris-HCl pH 7.4 and the pH was then readjusted to 7.4 using 5M NaOH and the solution was then made up to $100cm^3$ with 1M Tris-HCl to produce the respective concentrations.

Table 2.14. Quantities of ascorbic acid dissolved in $100cm^3$ to produce the required ascorbic acid standards.

Ascorbic Acid / mol dm^{-3}	Ascorbic Acid / g
0.01	0.1760
0.02	0.3523
0.03	0.5284
0.04	0.7045
0.05	0.8800
0.06	1.0570
5×10^{-3}	0.0882

Each of the aliquots of 5×10^{-3}M ascorbic acid listed in Table 2.15 were diluted to $90cm^3$ with 1M Tris-HCl pH 7.4, the pH was readjusted to 7.4 using 5M NaOH, and each of these solutions were then made up to $100cm^3$ with 1M Tris-HCl pH 7.4 to produce the respective ascorbic acid concentrations.

Table 2.15. Aliquots of ascorbic acid diluted to 100cm^3 to produce the required ascorbic acid standards.

Ascorbic Acid / mol dm^{-3}	5 x 10^{-3}M Ascorbic Acid / cm^3
5 x 10^{-4}	10
1 x 10^{-3}	20
2 x 10^{-3}	40

3. Potassium Dichromate Standards.

(a). 3.7 x 10^{-4}M $K_2Cr_2O_7$.

0.0105g of $K_2Cr_2O_7$ were dissolved in 100cm^3 of distilled water.

(b). 5.0 x 10^{-4}M $K_2Cr_2O_7$.

0.0142g of $K_2Cr_2O_7$ were dissolved in 100cm^3 of distilled water.

(c). 1 x 10^{-3}M $K_2Cr_2O_7$.

0.0283g of $K_2Cr_2O_7$ were dissolved in 100cm^3 of distilled water.

(d). 2 x 10^{-3}M $K_2Cr_2O_7$.

0.0567g of $K_2Cr_2O_7$ were dissolved in 100cm^3 of distilled water.

2.5.1.(c). Instrumentation.

PH meter, Jenway, Lennox, Model 3310.

U.V.-1601, UV-Visible Spectrophotometer, Schimadzu.

Expandable Ion Analyzer EA 920, Orion Research.

Platinum Wire Electrode.

Orion Double Junction Reference Electrode.

Computer - Memorex Telex.

Strawberry Tree Quick_Log Data Acquisition Software.

2.5.1.(d). Procedure for the Reduction of Cr(VI) by Ascorbic Acid.

One of the ascorbic acid standards above, 0.01M – 0.06M was heated to 25°C in a water bath as was the 3.7 x 10^{-3}M $K_2Cr_2O_7$. Following this a 2cm^3 aliquot of the ascorbic acid standard was added to 2cm^3 of the 3.7 x 10^{-4} $K_2Cr_2O_7$ in a plastic cuvette. Immediately after addition the solution was shaken and the absorbance of the Cr(VI) at 375 nm was recorded over the desired period of time (240 s). Instrument parameters were set as follows:

Kinetics.

1. Meas Mode - Abs.
2. Meas Time - 240 seconds
   Lag Time - 0 seconds.
   Rate Time - 10 seconds.
3. Factor - 1.000
4. Rec Range - 0.00A – 2.00A.
5. Temp Control - 25°C.
6. Time Scale - seconds.

Then by pressing the START button the absorbance was recorded. The temperature was maintained at 25°C throughout by using a thermostated cell. When the experiment was complete - RETURN, MODE and then DATA PROCESSING (6) were pressed. From here POINT PICK (6) was selected and the parameters were set as START POINT: 0.0 and INTERVAL: 10.0. This gave the absorbance reading for the reduction of Cr(VI) by ascorbic acid every 10 seconds. From these points a graph of absorbance verses time/seconds was obtained for each of the ascorbic acid standards. It was observed that the rate at which the absorbance measurements

decreased with time increased as the ascorbic acid concentration increased. Figures 2.49-2.54 represent the decrease in absorbance measurements that were obtained from these experiments. The data is given in appendix 2.42-2.47 respectively. Each of these graphs were then fitted to the function A*exp(-b*x), and the pseudo-first-order-rate constant, (B value = K_{obs}) was obtained from each graph.

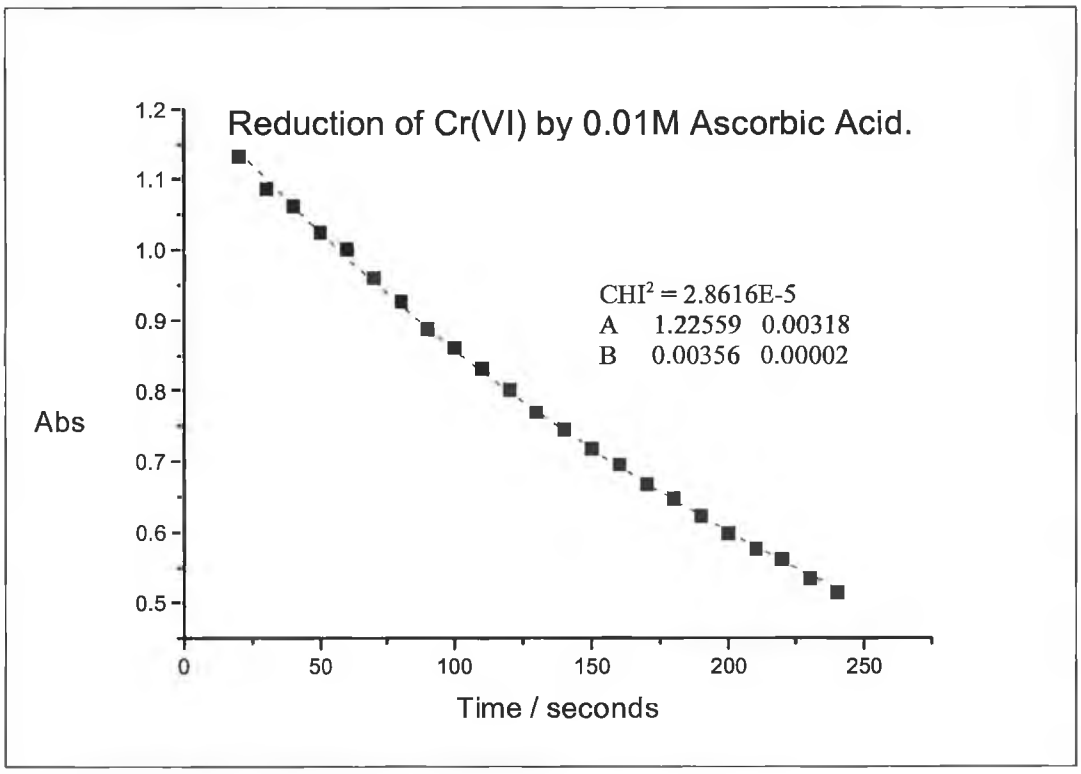


Figure 2.49. Reduction of Cr(VI) by 0.01M Ascorbic Acid.

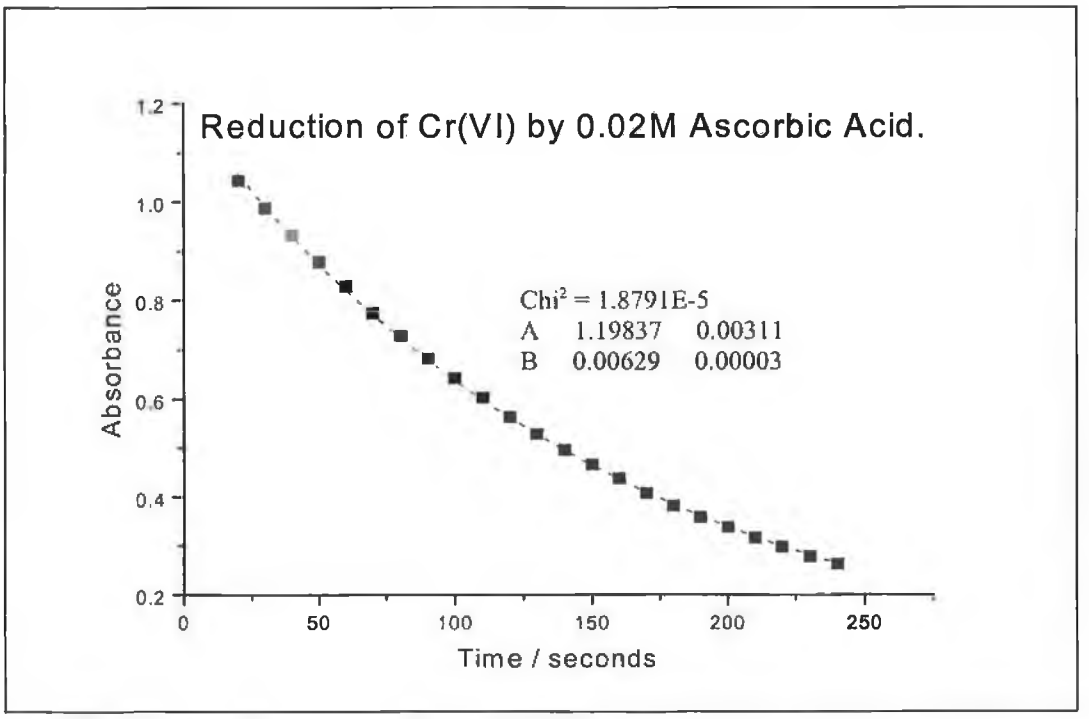


Figure 2.50. Reduction of Cr(VI) by 0.02M Ascorbic Acid.

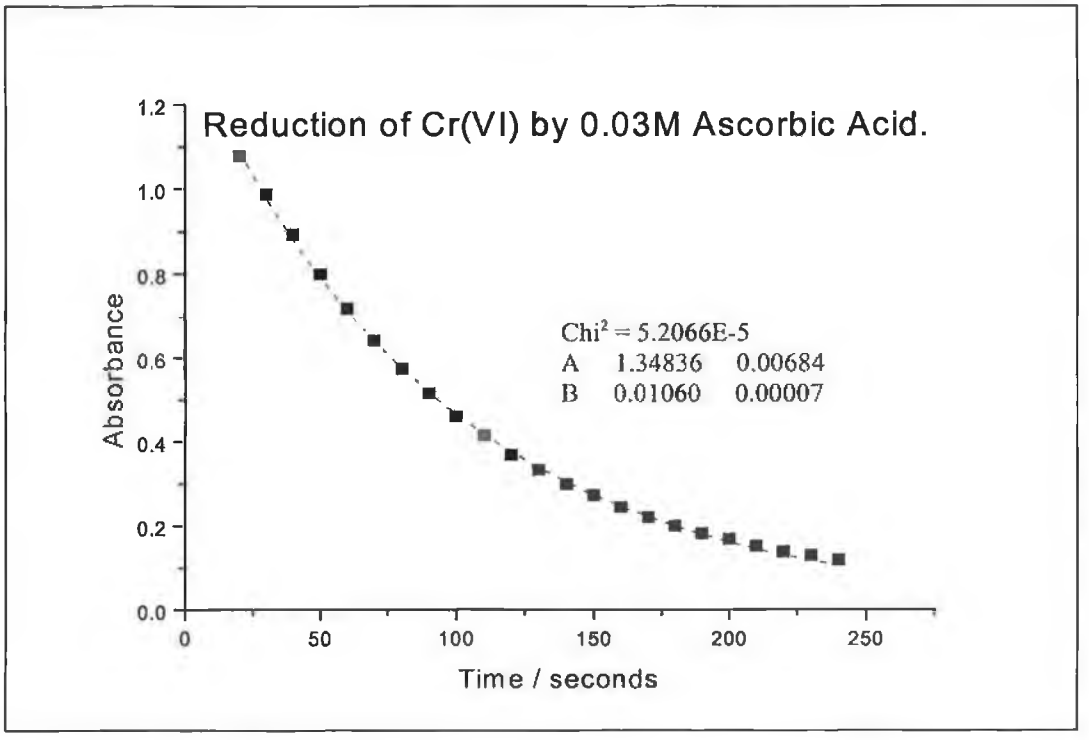


Figure 2.51. Reduction of Cr(VI) by 0.03M Ascorbic Acid

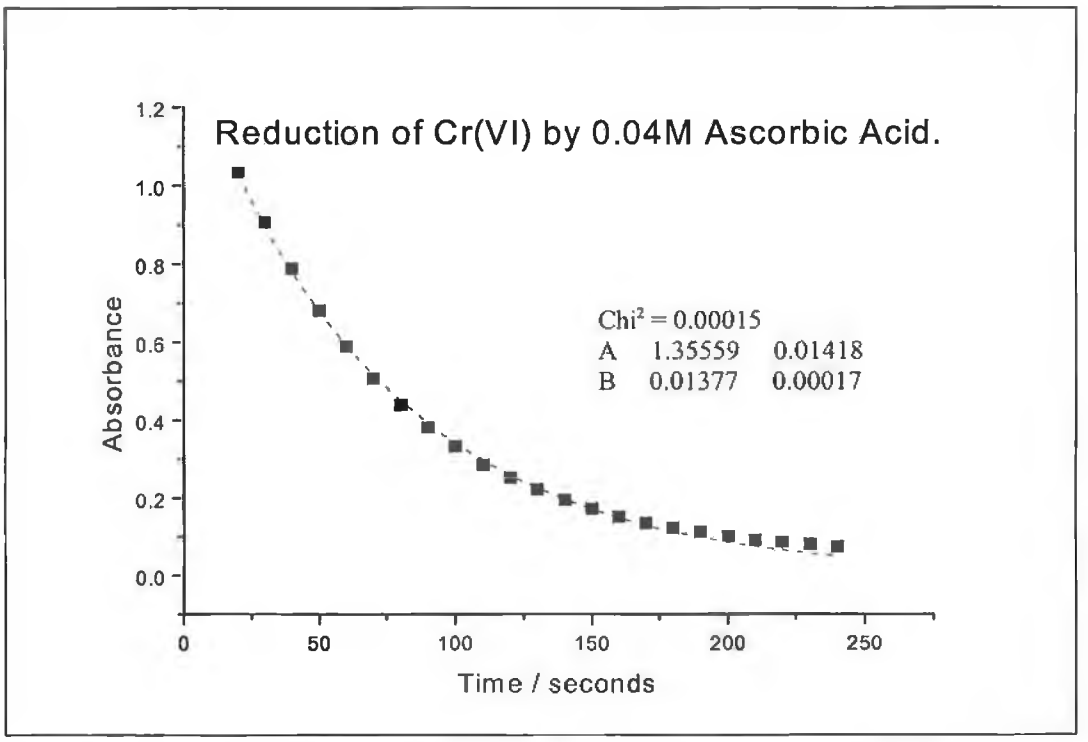


Figure 2.52. Reduction of Cr(VI) by 0.04M Ascorbic Acid.

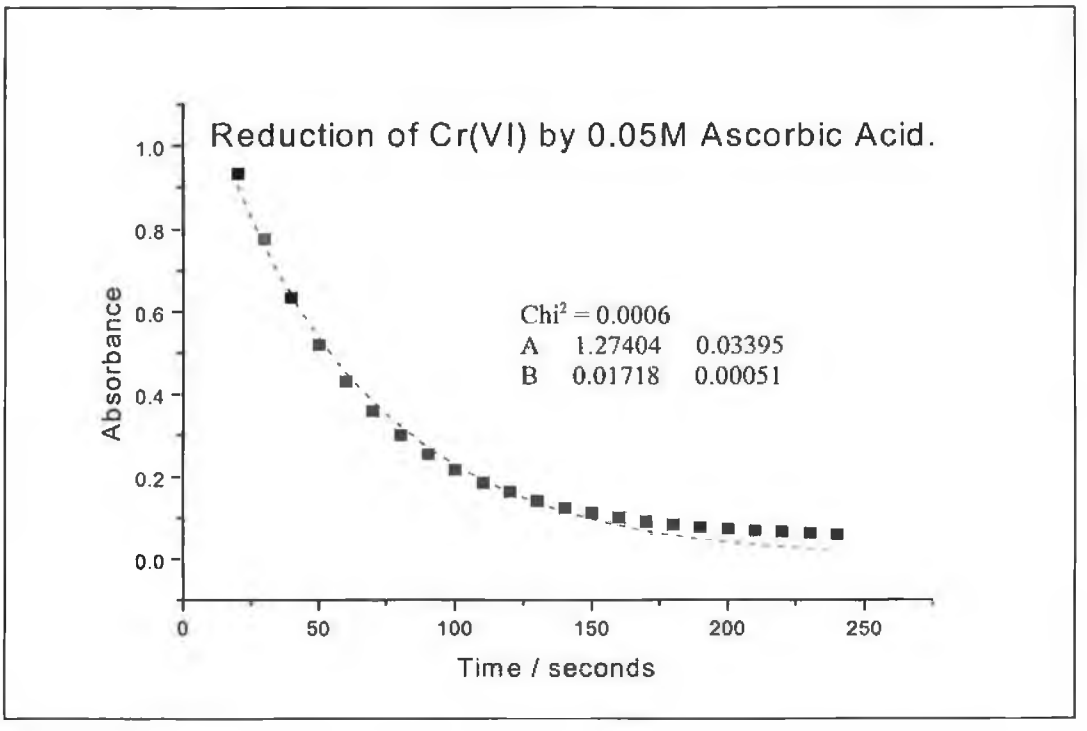


Figure 2.53. Reduction of Cr(VI) by 0.05M Ascorbic Acid.

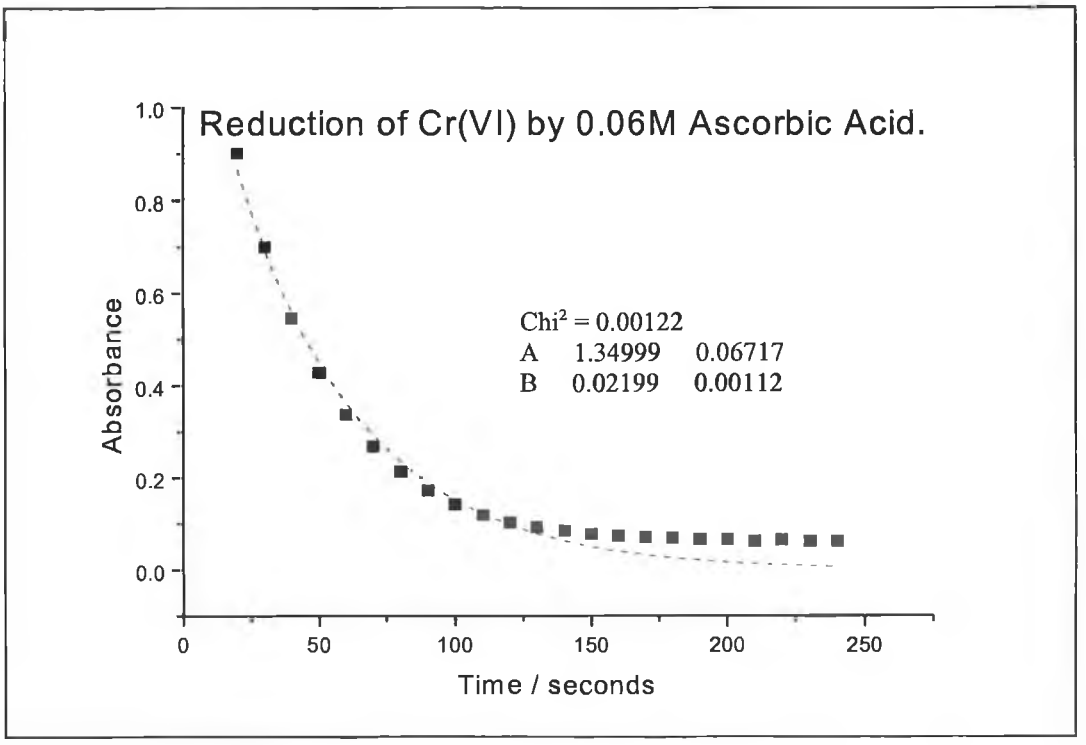


Figure 2.54. Reduction of Cr(VI) by 0.06M Ascorbic Acid.

Table 2.16. Pseudo-First-Order-Rate Constants obtained form above graphs.

Concentration of Ascorbic Acid / mol dm^{-3}	Pseudo-First-Order-Rate Constant / seconds $^{-1}$
0.01	0.00356
0.02	0.00629
0.03	0.01060
0.04	0.01337
0.05	0.01718
0.06	0.02199

From these points the pseudo-first-order-rate constant (K_{obs}) verses ascorbic acid concentration/mol dm^{-3} was plotted and this gave a straight line implying that the reaction was first order with respect to ascorbic acid concentration. Figure 2.55

represents the straight line obtained from the K_{obs} values v's ascorbic acid concentration and the data is given in appendix 2.48. From this the second rate constant was obtained from the slope of the graph and was found to be 0.366 dm^3 mol^{-1} s^{-1}.

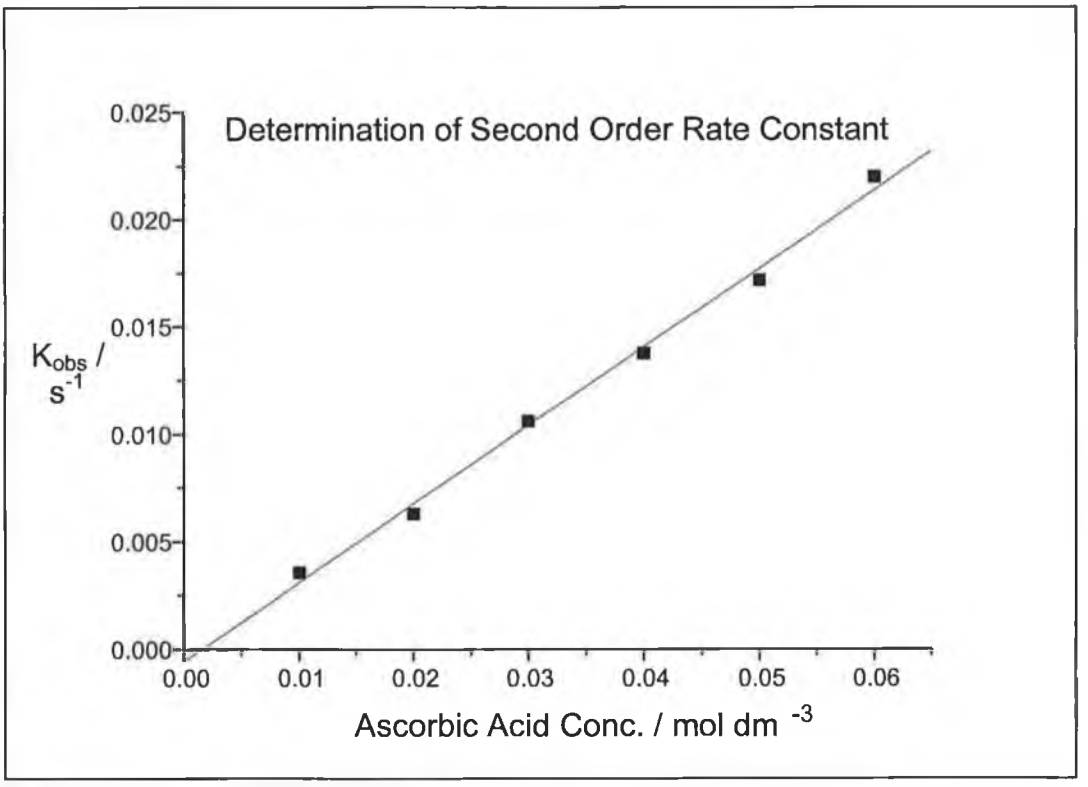


Figure 2.55. Determination of the second order rate constant.

Table 2.17. Data from the graph of the determination of the second order rate constant.

Parameter	Value
A	-0.00057±0.00051
B	0.36569±0.01320
R	0.9974
SD	0.00055
N	6
P	0.00001

2.1.5.(e). The Effect of Anaerobic Conditions.

Dixon and Sadler (1993), reported that under anaerobic conditions L-ascorbic acid rapidly reduces chromium (VI) at a rate which is about 10 times faster than the rate of reaction carried out in oxygen. But O'Brien and Woodbridge (1997) later showed that this reported oxygen-dependence was caused by the use of non-demetallated Tris-HCl buffer and was not an intrinsic feature of the redox reaction between chromium (VI) and ascorbic acid. In order to find out if the presence of air has any effect on the rate of reduction of Cr(VI) by ascorbic acid the following was carried out.

A $2cm^3$ aliquot of 0.03M ascorbic acid and a $2cm^3$ aliquot of 3.7×10^{-3}M $K_2Cr_2O_7$ were mixed in a quartz cuvette. Immediately after addition the solution was shaken and the spectrum of the solution was obtained every minute for a total of 8 minutes. Instrument parameters were entered as follows:

Spectrum

1. Meas Mode - Abs.
2. Scanning Range - 470nm – 350nm.
3. Rec. Range - 0.00A - 1.00A.
4. Scan Speed - Fast.
5. No. of Scans - 8 scans. Int: 60 sec.
6. Display Mode - Overlay.

After the 8 minutes had elapsed and all the spectra were recorded a print out of the spectra were obtained and it was found that most of the Cr(VI) had been reduced by the ascorbic acid in 6 minutes. The spectra obtained from this experiment are shown in figure 2.56.(a).

Following this the solution of the 0.03M ascorbic acid was deaerated for 10 minutes using nitrogen as was the solution of the 3.7 x 10^{-3}M $K_2Cr_2O_7$. A $2cm^3$ aliquot of both of these solutions were then mixed and immediately a cap was placed on top of the quartz cell to prevent any air from entering the solution. Then this solution was analysed in the same way as the solutions under aerobic conditions, and again a spectrum was recorded every minute for 8 minutes and a print out was obtained. Figure 2.56.(b) represents the spectra obtained for this experiment. Again it was found that most of the Cr(VI) had been reduced within 6 minutes.

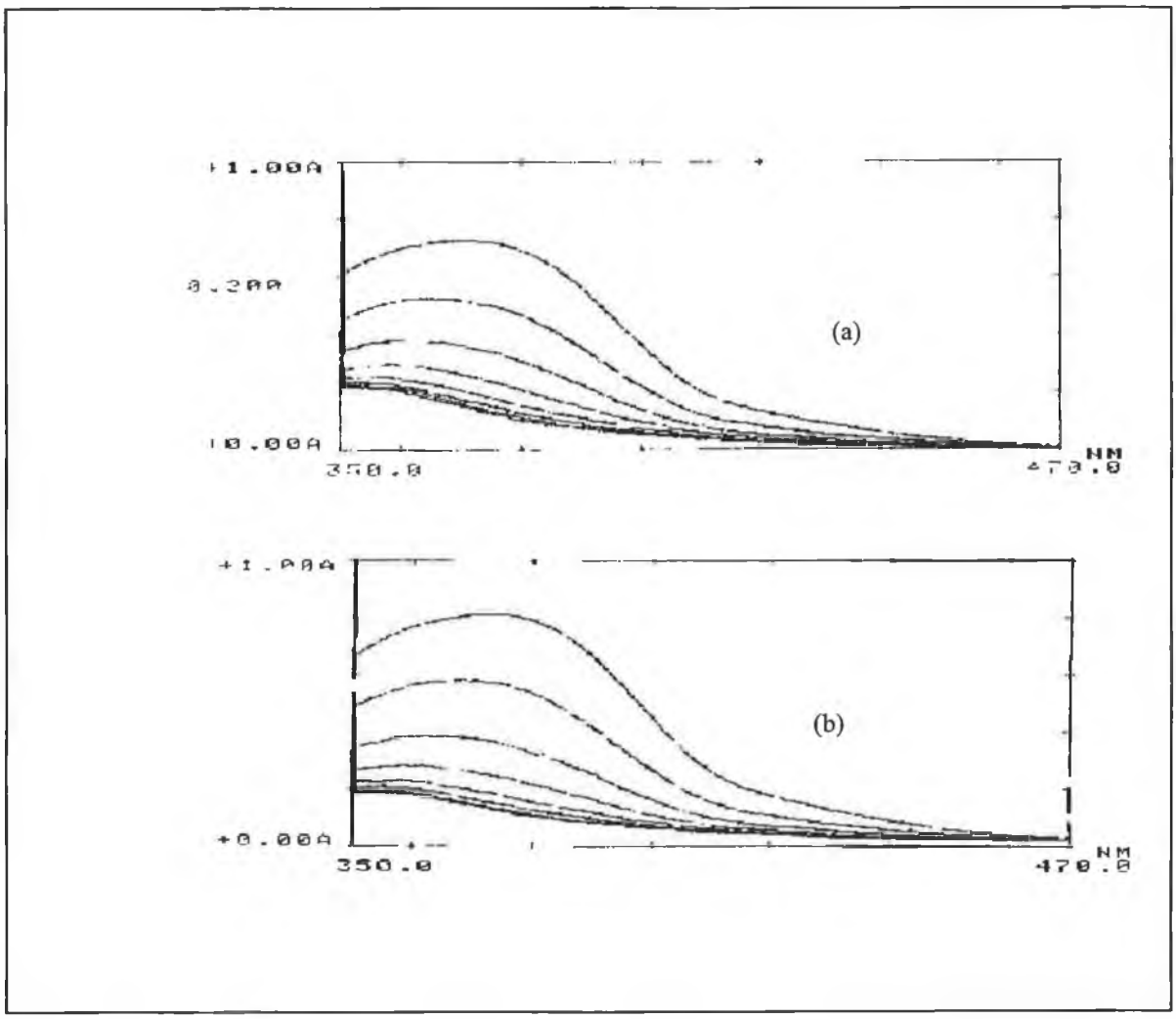


Figure 2.56. Spectra obtained for the reduction of Cr(VI) by Ascorbic Acid in (a) aerobic conditions and (b) anaerobic conditions.

On comparing the spectra of each of the solutions above, (a) in aerobic conditions and (b) in anaerobic conditions, they appear to be quite similar, and the absence of air appeared to have little if any effect on the rate of reduction of Cr(VI) by ascorbic acid i.e. the rate of reduction of Cr(VI) by ascorbic acid over 8 minutes was the same regardless of aerobic or anearobic conditions.

2.5.2. Reduction Capacity of Chromium Enriched Yeast.

The reduction of Cr(VI) by excess chromium enriched yeast in acid, at pH 7.4 and at pH 13 was studied by following the change in absorbance of the Cr(VI) peak at 375nm. The reactions were carried out in air and under nitrogen.

2.5.2.(a). Reagents and Solutions.

1. Trizma (Tris[hydroxymethyl]aminomethane) hydrochloride, $C_4H_{11}NO_3.HCl$, F.W.=157.6g mol^{-1}, Sigma, #T3254.
2. Potassium Dichromate, $K_2Cr_2O_7$, F.W. = 294.49g mol^{-1}, Wardle Chemical Company Ltd., #PO4868.
3. Sulphuric Acid, H_2SO_4, S.G. = 1.84, F.W. = 98.07g mol^{-1}, Romil Ltd., Code A-9691.
4. Tris(hydroxymethyl)aminomethane, $C_4H_{10}NO_3$, F.W.= 121.12g mol^{-1}, Riedel-de Haen, #33742.
5. Chromium Enriched Yeast, Alltech Ireland, Dunboyne, Co. Meath, Ref. 158233.
6. Non-Enriched Yeast, Active Dried Yeast, DCL Yeast Limited.

Preparation of Solutions.

1. 1M Tris-HCl pH 7.4.

As is section 2.5.2.

2. H_2SO_4 Standards.

(a). 1M H_2SO_4.

54.54cm^3 of concentrated H_2SO_4 was diluted to 1 dm^3 using distilled water.

(b). 0.1M H_2SO_4.

100cm^3 of 1M H_2SO_4 were diluted to 1 dm^3 with distilled water.

(c). 0.01M H_2SO_4.

100cm^3 of 0.1M H_2SO_4 were diluted to 1 dm^3 with distilled water.

3. *Tris-NaOH pH 13 Standards.*

(a). 0.2M Tris-NaOH pH 13.

As in section 2.1.3.(b).

(b). 0.1M Tris-NaOH pH 13.

50cm^3 of 0.2M Tris-NaOH pH 13 were diluted to 100cm^3 with distilled water.

4. *Potassium Dichromate Standards.*

(a). 1000ppm Cr(VI).

As in section 2.1.1.(b).

(b). 100ppm Cr(VI).

As in section 2.1.3.(b).

(c). 1ppm, 0.1ppm, and 0.01ppm Cr(VI).

As in section 2.3.2.(a).

2.5.2.(b). Experimental Procedures.

To 100cm^3 aliquots of (a) 1M H_2SO_4, (b) 1M Tris-HCl pH 7.4, and (c) 0.2M Tris-NaOH pH 13 buffer the quantities of potassium dichromate listed in Table 2.11.(a), (b) and (c) were added and dissolved. Chromium enriched yeast was then added (exact quantities are listed in Table 2.18.(a), (b) and (c)) and 5cm^3 extracts were taken from these solutions at various time intervals, filtered through a 0.2μm syringe filter and the absorbance measurement of each solution was obtained at 375nm using U.V. spectroscopy.

Table 2.18.(a). Quantities of $K_2Cr_2O_7$ and Chromium Enriched Yeast added to 1M HCl pH1.

Cr(VI) / ppm	Mass of $K_2Cr_2O_7$ / g Air	Mass of $K_2Cr_2O_7$ / g Nitrogen	Mass of Enriched Yeast / g Air	Mass of Enriched Yeast / g Nitrogen
200	0.0567	0.0568	0.2501	0.2504
100	0.0285	0.0286	0.2503	0.2505
50	0.0143	0.0142	0.2504	0.2502

Table 2.18.(b). Quantities of $K_2Cr_2O_7$ and Chromium Enriched Yeast added to 1M Tris-HCl pH 7.4.

Cr(VI) / ppm	Mass of $K_2Cr_2O_7$ / g Air	Mass of $K_2Cr_2O_7$ / g Nitrogen	Mass of Enriched Yeast / g Air	Mass of Enriched Yeast / g Nitrogen
200	0.0569	0.0566	0.2499	0.2498
100	0.0284	0.0285	0.2507	0.2507
25	0.0072	0.0074	0.2503	0.2504

Table 2.18.(c). Quantities of $K_2Cr_2O_7$ and Chromium Enriched Yeast added to 0.2M Tris-NaOH pH 13.

Cr(VI) / ppm	Mass of $K_2Cr_2O_7$ / g Air	Mass of $K_2Cr_2O_7$ / g Nitrogen	Mass of Enriched Yeast / g Air	Mass of Enriched Yeast / g Nitrogen
200	0.0567	0.0566	0.2504	0.2502
100	0.0285	0.0282	0.2502	0.02507
25	0.0073	0.0071	0.2502	0.2507

The above procedure was carried out both in the presence of air and in the absence of air (i.e. under nitrogen). For the procedure carried out in the absence of air the potassium dichromate was dissolved in the buffer and then deaerated for 15 minutes. After the addition of the yeast deaeration was carried out again for 15 minutes, and the solution was kept under nitrogen at all times.

Results:

(a). For the procedure carried out in 1M H_2SO_4 (acidic solution), both in air and in nitrogen, the yeast continuously reduced the Cr(VI) that was present. The absence of air seemed to have little effect on the rate of reduction of the Cr(VI) by the chromium enriched yeast since the rate of reduction of Cr(VI) in the presence of air appeared to be very similar to that in the absence of air, for the 200, 100 and 50ppm Cr(VI) solutions. Figures 2.57-2.62 represent the reduction in absorbance measurements obtained over time and the data for these experiments is given in appendix 2.49-2.54.

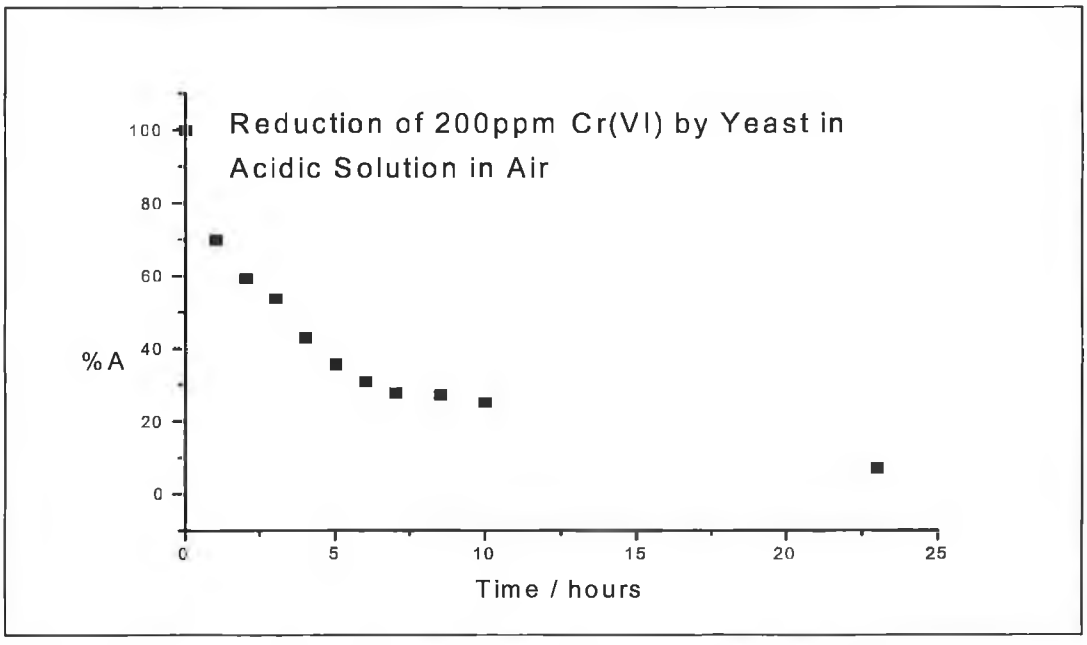


Figure 2.57. Reduction of 200ppm Cr(VI) by chromium enriched yeast in acidic solution in air.

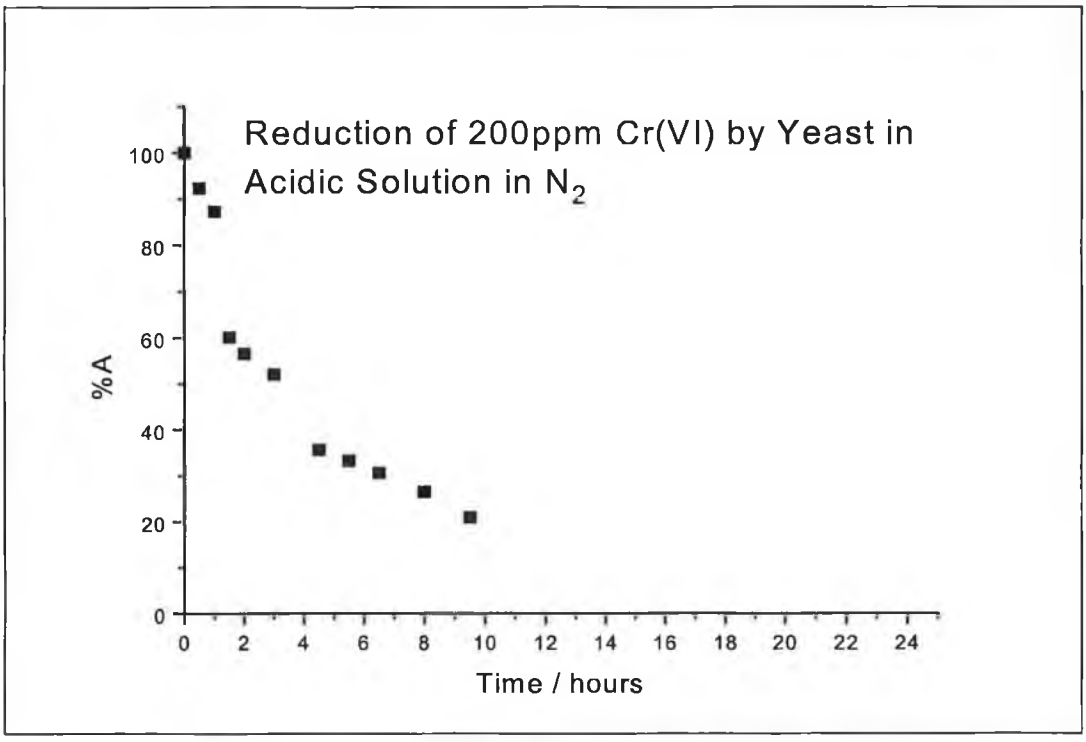


Figure 2.58. Reduction of 200ppm Cr(VI) by chromium enriched yeast in acidic solution in nitrogen.

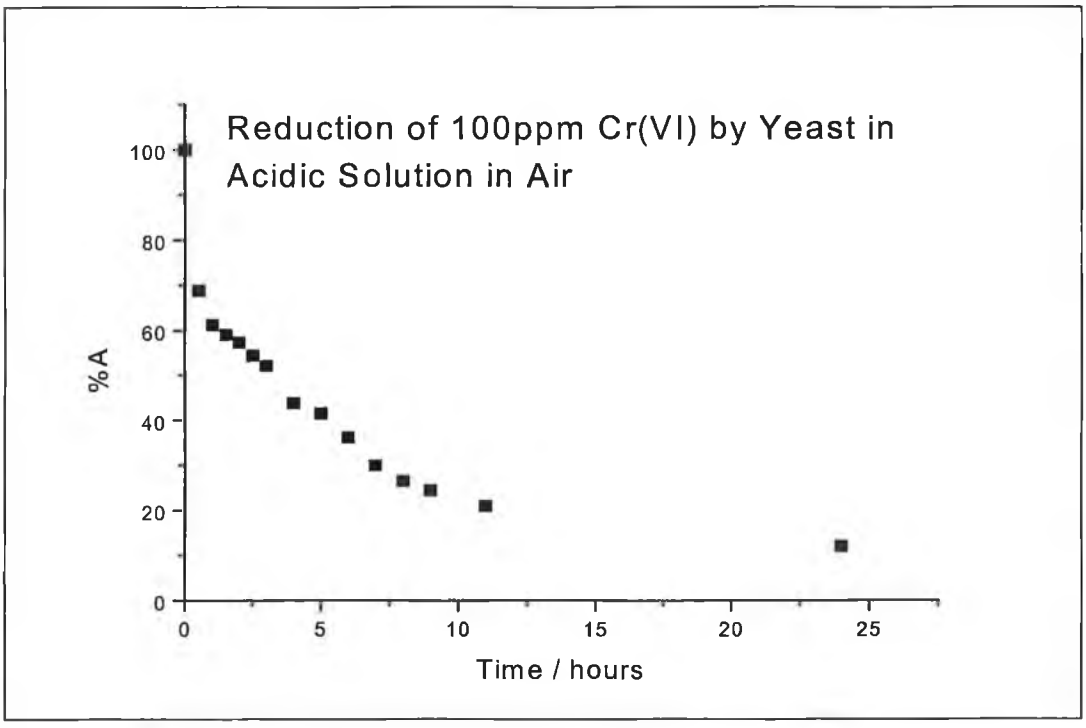


Figure 2.59. Reduction of 100ppm Cr(VI) by chromium enriched yeast in acidic solution in air.

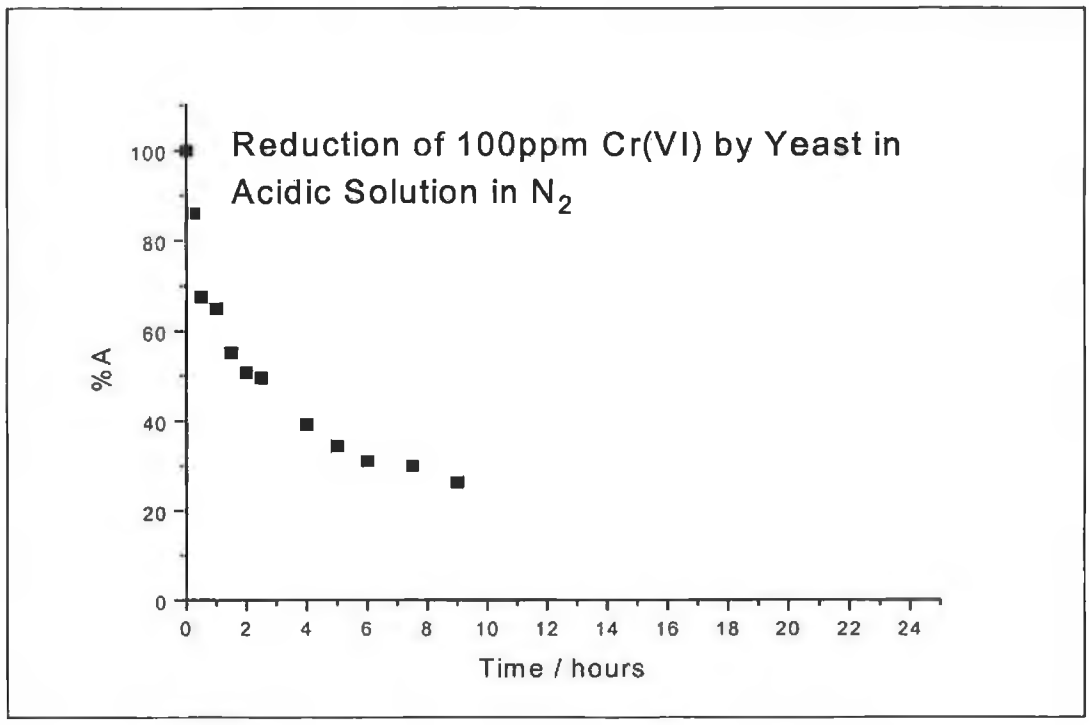


Figure 2.60. Reduction of 100ppm Cr(VI) by chromium enriched yeast in acidic solution in nitrogen.

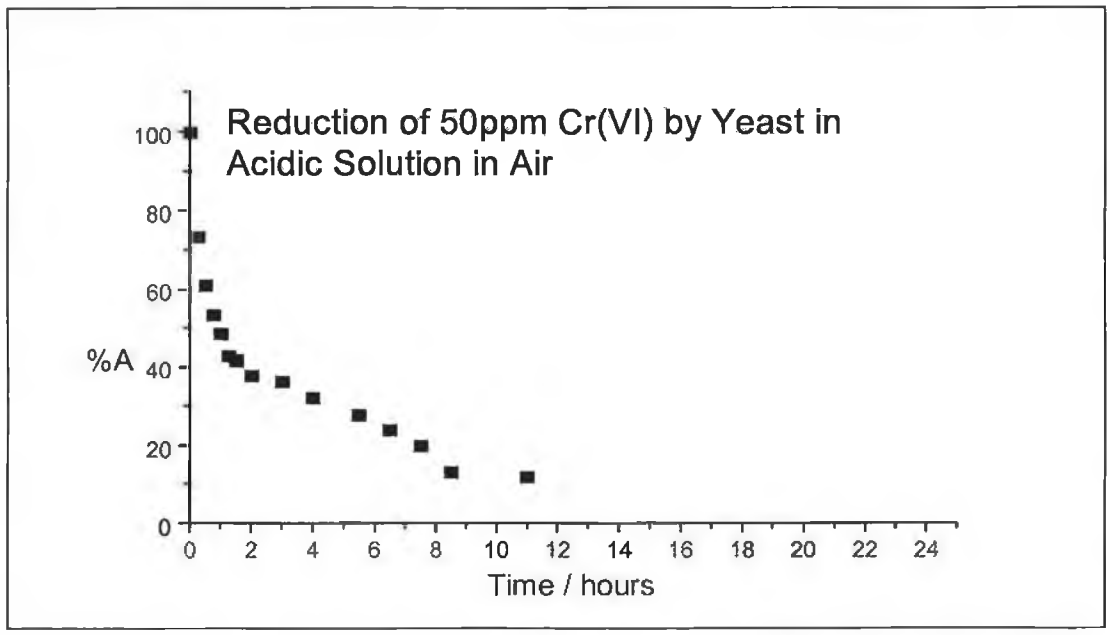


Figure 2.61. Reduction of 50ppm Cr(VI) by chromium enriched yeast in acidic solution in air.

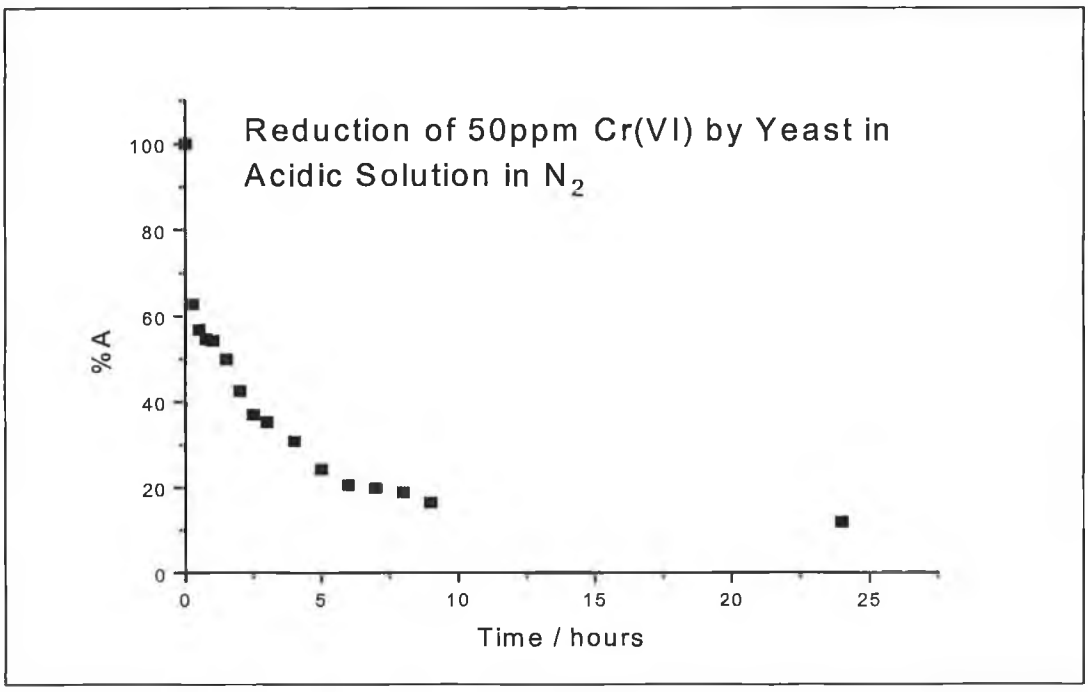


Figure 2.62. Reduction of 50ppm Cr(VI) by chromium enriched yeast in acidic solution in nitrogen.

(b). For the procedure carried out in 1M Tris-HCl pH 7.4 (neutral solution), both in air and in nitrogen, for all solutions 200, 100 and 25 ppm Cr(VI), initially the enriched yeast appeared to reduce the Cr(VI) that was present, but over time oxidation occurred and the %A increased again. Following this, reduction and oxidation of Cr(VI) occurred periodically over time for all the solutions. Figures 2.63-2.68 represent the absorbance measurements obtained over time and the data for these experiments is given in appendix 2.55-2.60.

The %A is the absorbance at time as a percentage of the initial absorbance.

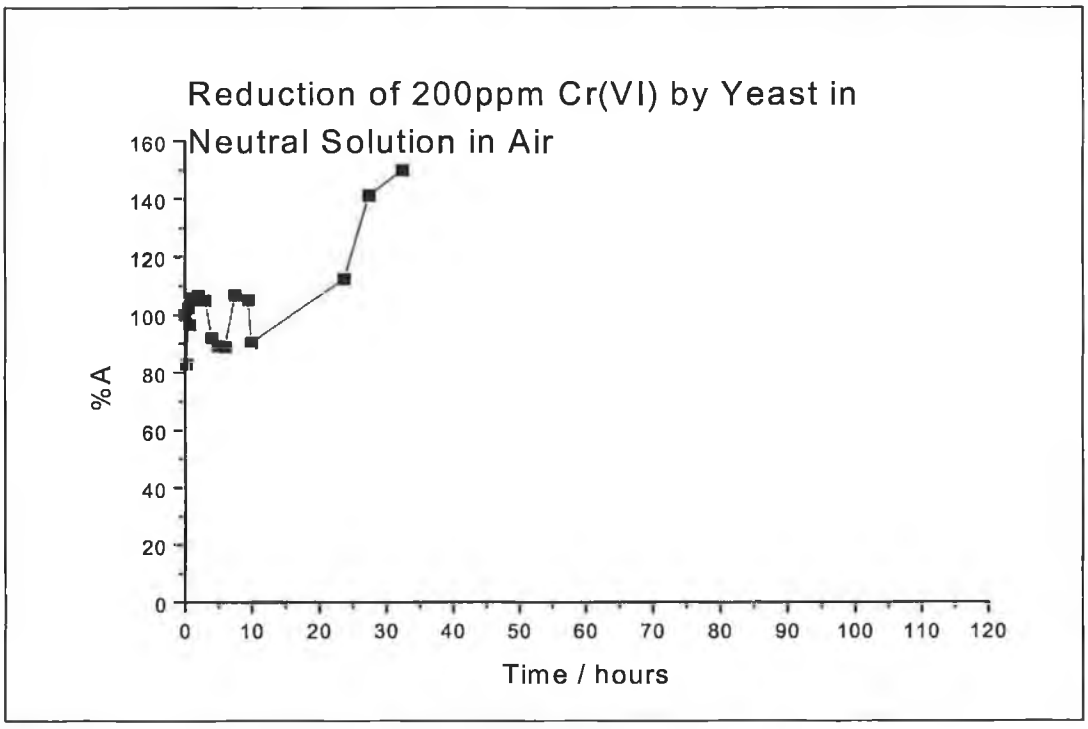


Figure 2.63. Reduction of 200ppm Cr(VI) by chromium enriched yeast in neutral solution in air.

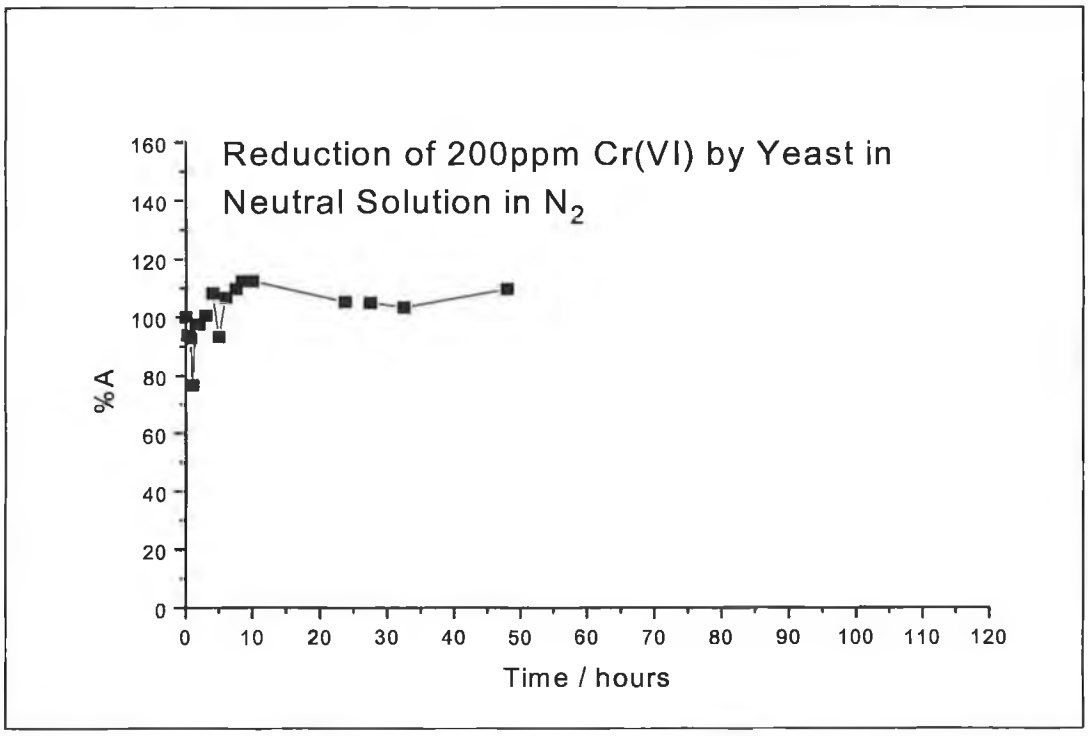


Figure 2.64. Reduction of 200ppm Cr(VI) by chromium enriched yeast in neutral solution in nitrogen.

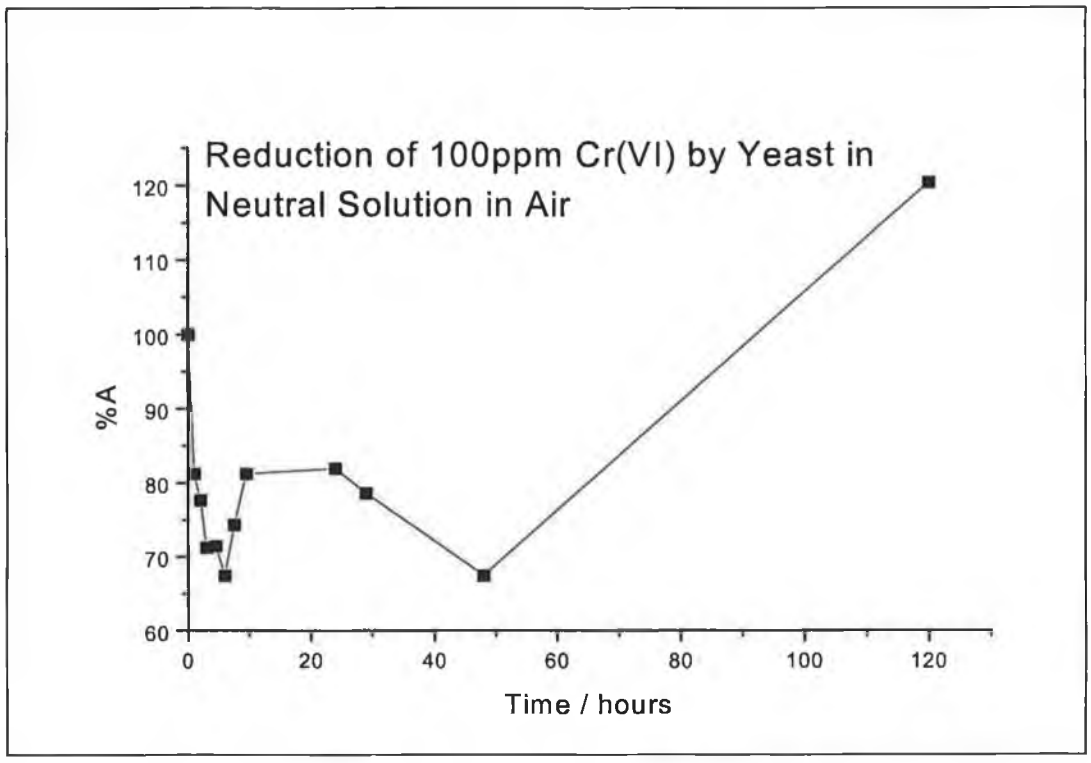


Figure 2.65. Reduction of 100ppm Cr(VI) by chromium enriched yeast in neutral solution in air.

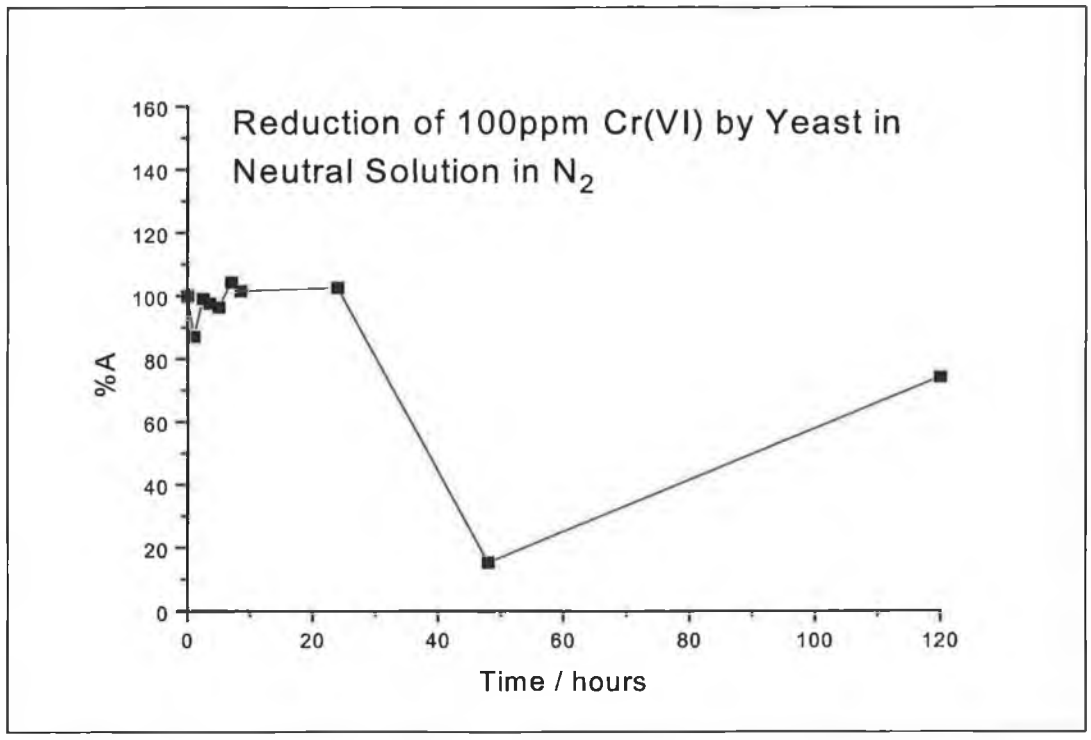


Figure 2.66. Reduction of 100ppm Cr(VI) by chromium enriched yeast in neutral solution in nitrogen.

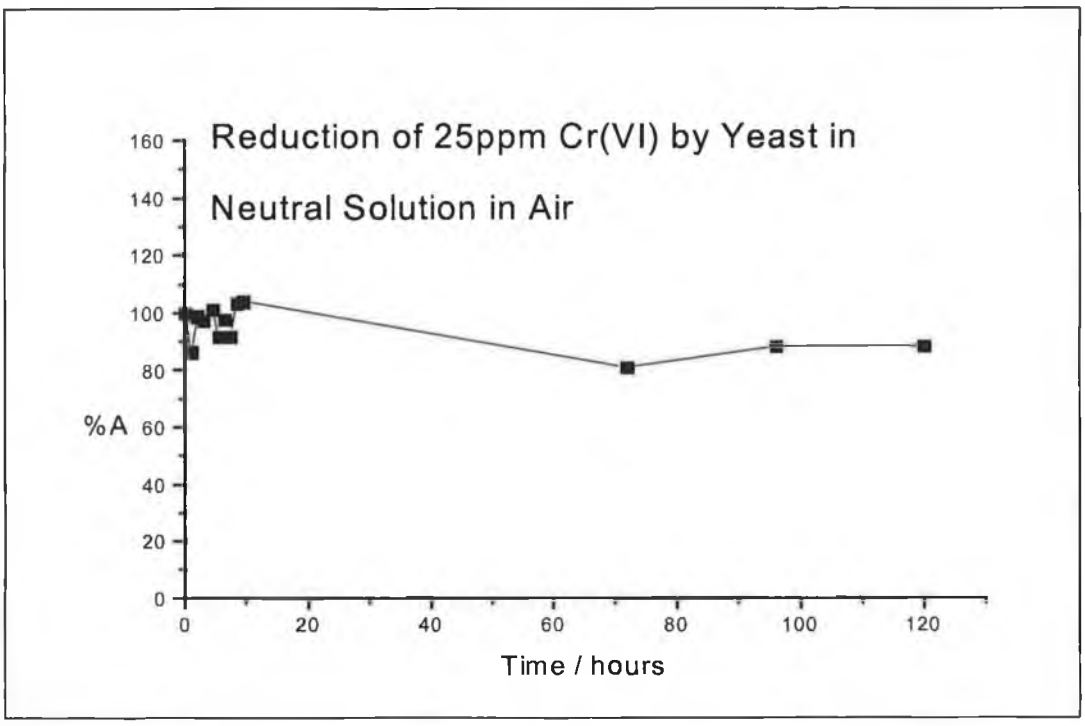


Figure 2.67. Reduction of 25ppm Cr(VI) by chromium enriched yeast in neutral solution in air.

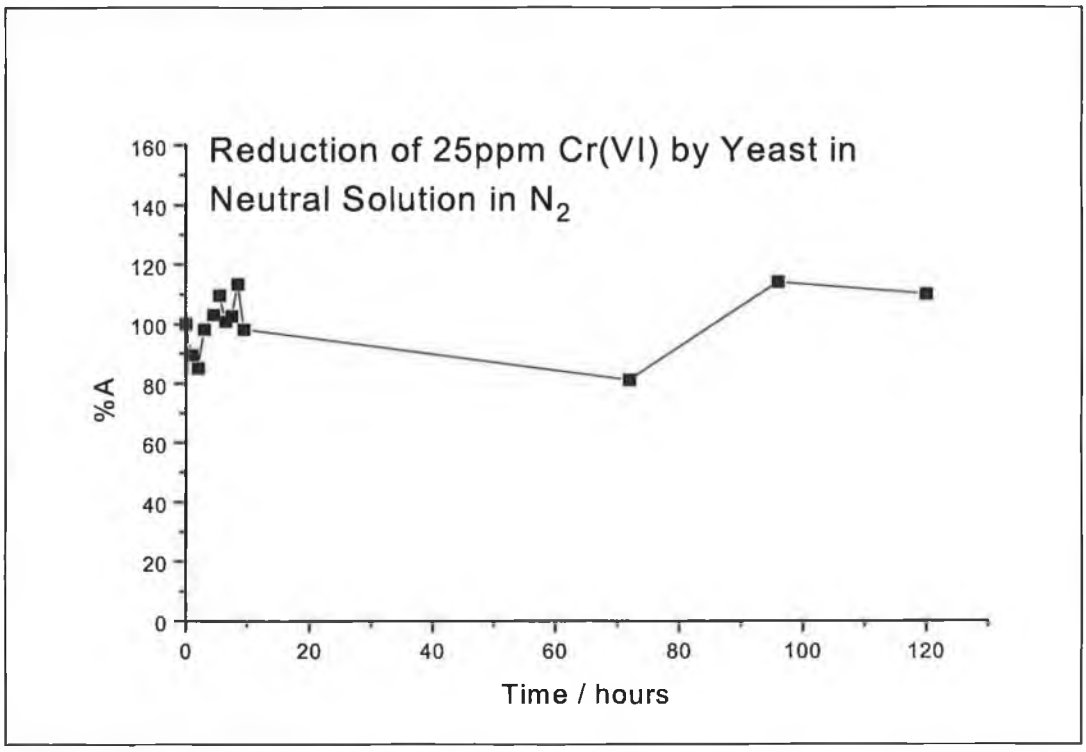


Figure 2.68. Reduction of 25ppm Cr(VI) by chromium enriched yeast in neutral solution in nitrogen.

(c). For the procedure carried out in 0.2M Tris-NaOH pH 13 (basic solution), both in air and in nitrogen for all solutions, 200, 100 and 25ppm Cr(VI), again initially the enriched yeast reduced the Cr(VI) but again over time oxidation occurred and the %A increased. This reduction and oxidation of Cr(VI) occurred periodically over time. Figures 2.69-2.74 represent the absorbance measurements obtained over time and the data for these experiments is given in appendix 2.61-2.66.

From the results obtained from the above experiments it can be seen that the chromium enriched yeast will reduce Cr(VI), even as high as 200ppm, in less then 24 hours in acid solution. This is so because in acid solution Cr(VI) is present as the dichromate ion $Cr_2O_7^{2-}$, which is highly reactive and so readily reacted with organic matter present in the chromium enriched yeast and so it was reduced. In neutral and basic solutions Cr(VI) is present as the hydrogenchromate ion, $HCrO_4^-$ and the chromate ion, CrO_4^{2-} respectively, which are not as oxidising as the dichromate ion. Also Cr(III) is more readily oxidised to Cr(VI) in neutral and in alkaline solution.

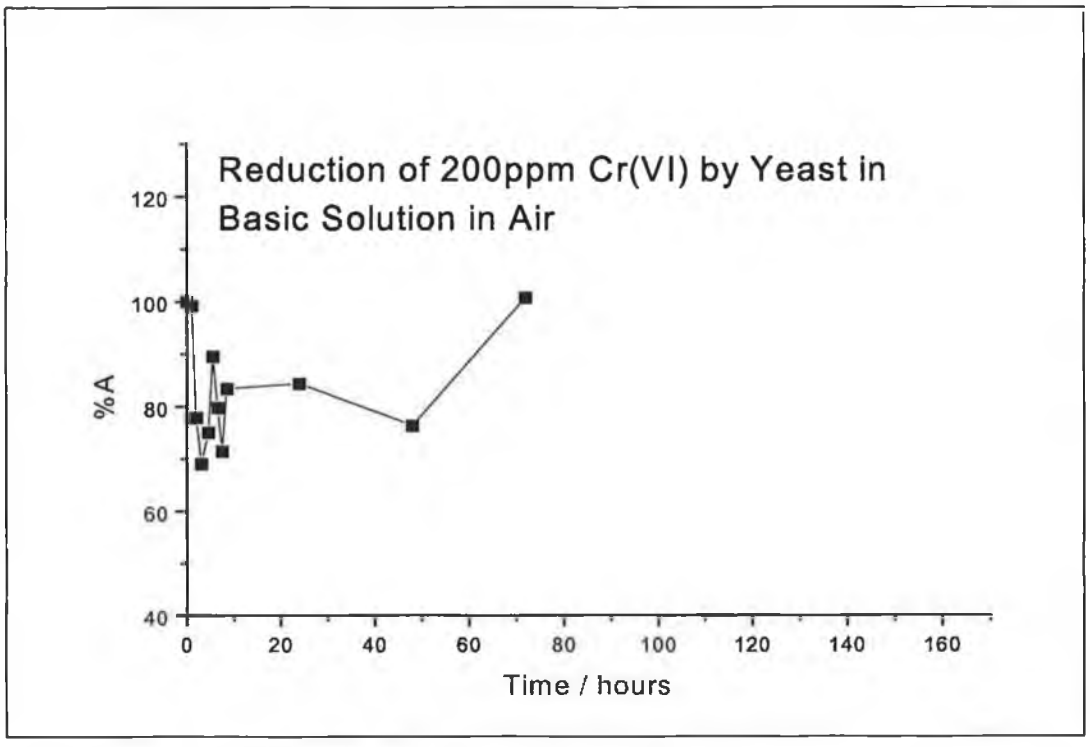


Figure 2.69. Reduction of 200ppm Cr(VI) by chromium enriched yeast in basic solution in air.

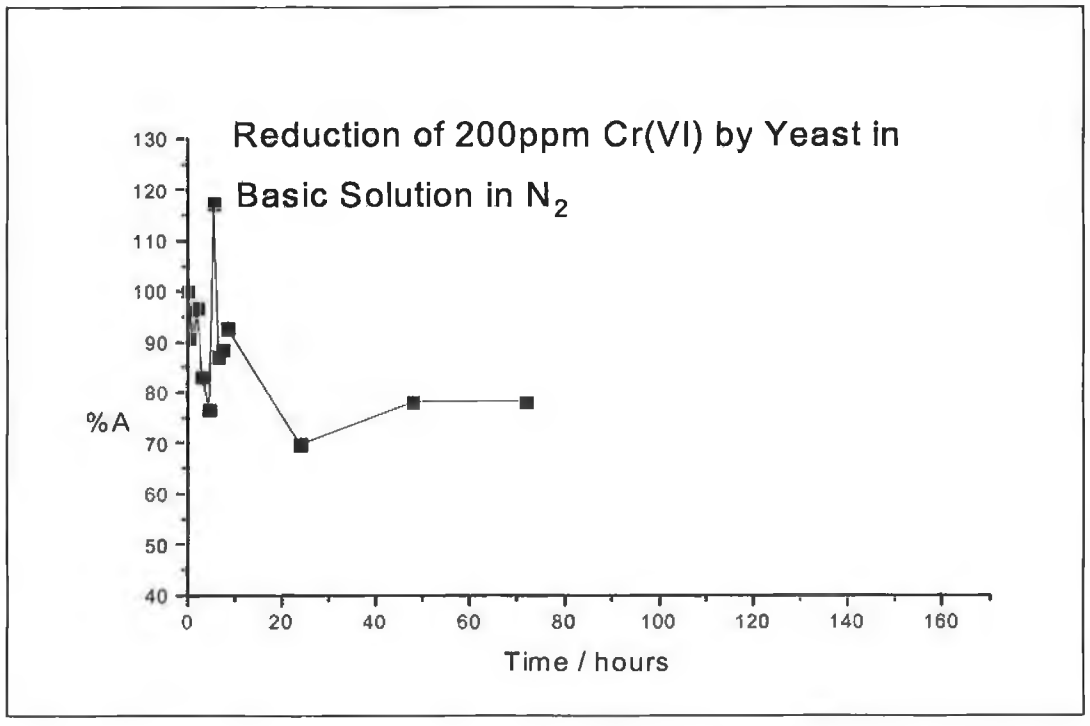


Figure 2.70. Reduction of 200ppm Cr(VI) by chromium enriched yeast in basic solution in nitrogen.

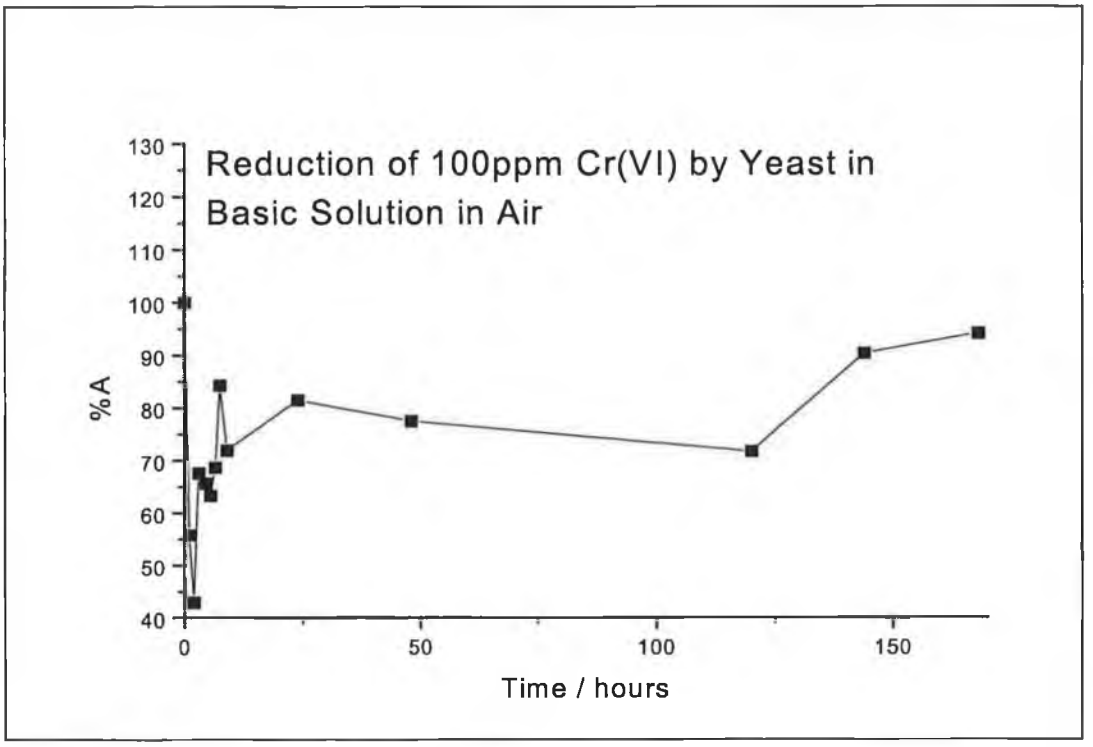


Figure 2.71. Reduction of 100ppm Cr(VI) by chromium enriched yeast in basic solution in air.

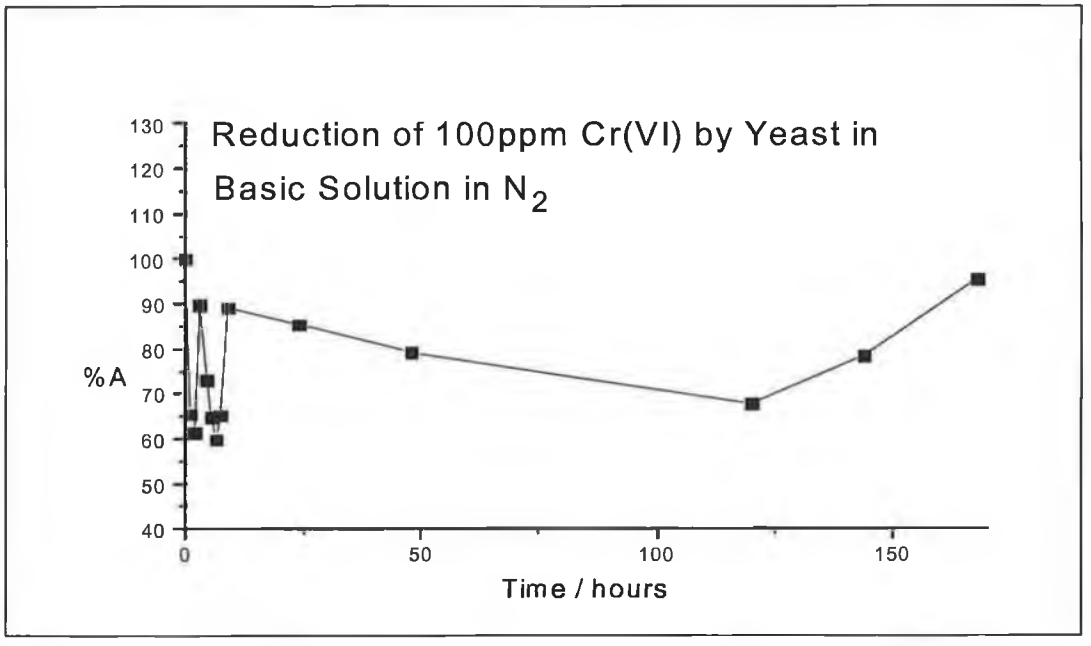


Figure 2.72. Reduction of 100ppm Cr(VI) by chromium enriched yeast in basic solution in nitrogen.

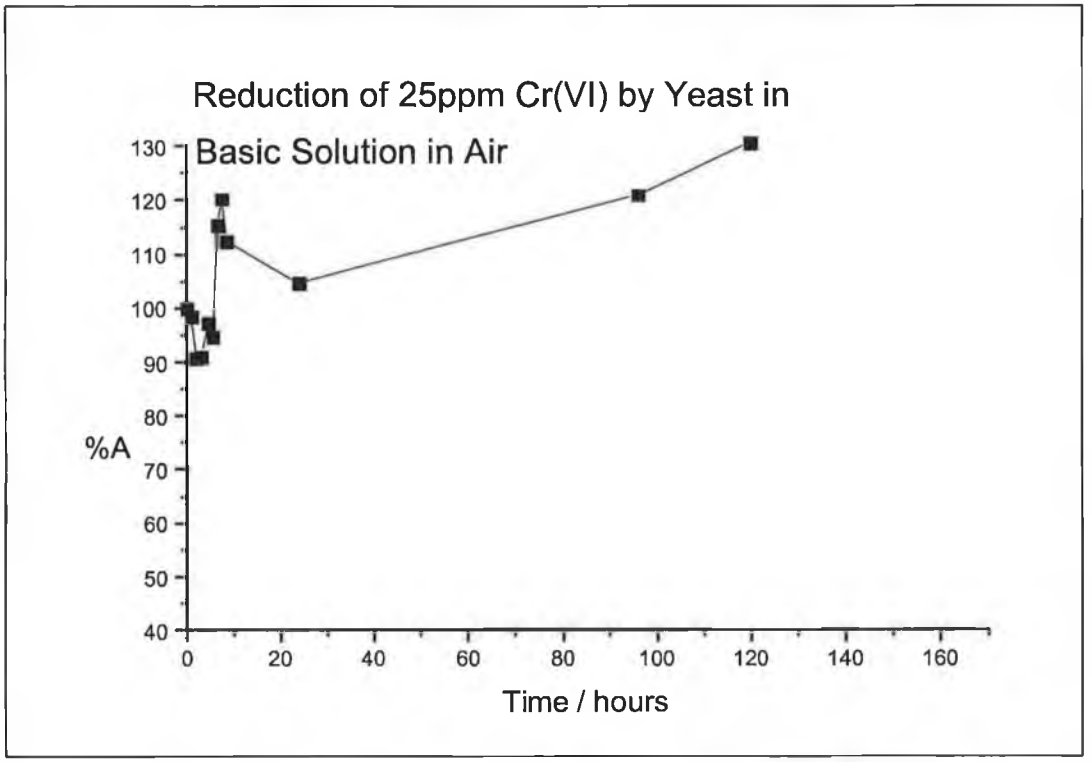


Figure 2.73. Reduction of 25ppm Cr(VI) by chromium enriched yeast in basic solution in air.

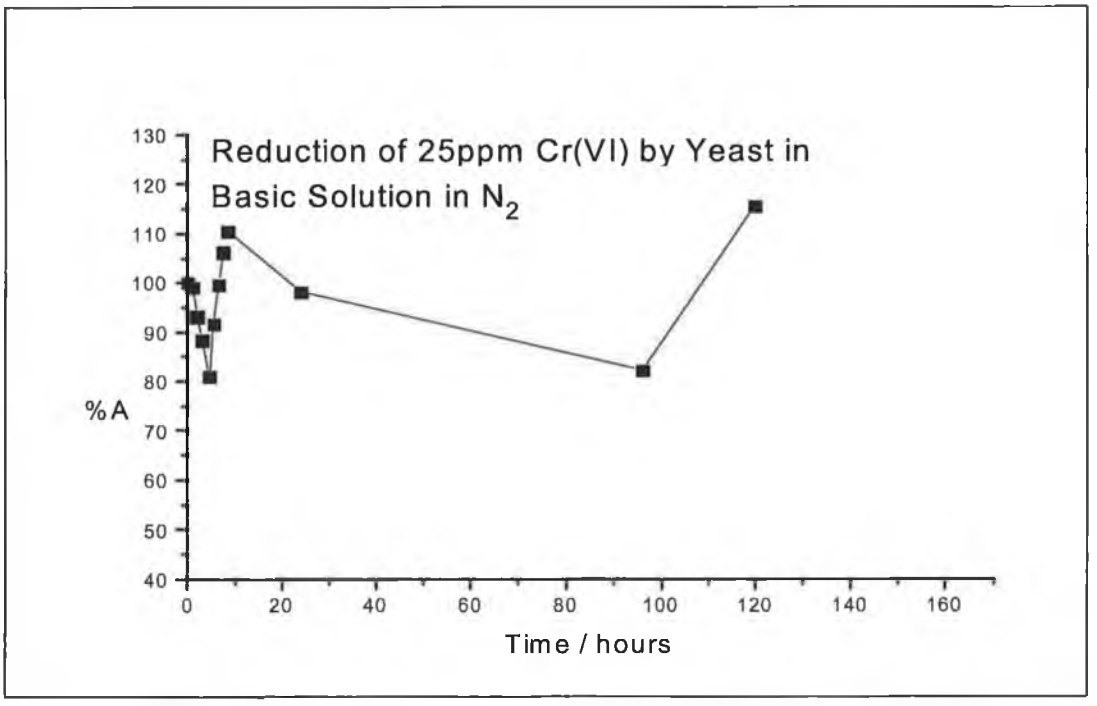


Figure 2.74. Reduction of 25ppm Cr(VI) by chromium enriched yeast in basic solution in nitrogen.

2.5.2.(c). Reduction Capacity of Non-Enriched Yeast.

This procedure was carried out in the same way for the acidic, neutral and basic buffer as is was for the experimental procedures for the chromium enriched yeast above, with the exception that only 100ppm Cr(VI) was added and that non-enriched yeast was added. The exact quantities added are listed in Table 2.19.

Table 2.19. Quantities of Cr(VI) and non enriched yeast required to produce 100ppm Cr(VI) in the respective buffers.

Cr(VI)/ppm	Buffer	Mass of $K_2Cr_2O_7$/g Air	Mass of $K_2Cr_2O_7$/g Nitrogen	Mass of Non-Enriched Yeast/g Air	Mass of Non-Enriched Yeast/g Nitrogen
100	Acidic	0.0282	0.0282	0.2501	0.2502
100	Neutral	0.0280	0.0283	0.2501	0.2502
100	Basic	0.0282	0.0284	0.2498	0.2502

Result:

(a). For the procedure carried out in acidic solution, for both the solutions carried out in air and in nitrogen, the yeast reduced the Cr(VI) present and the absence of air appeared to have little effect on the rate of reduction. Figure 2.75 and 2.76 represent the absorbance measurements obtained for these experiments and the data is given in appendix 2.67 and 2.68.

(b). For the procedure carried out in neutral solution, initially there was reduction of the Cr(VI) and again oxidation and reduction occurred periodically over time. Figure 2.77 and 2.78 represent the absorbance measurements obtained for these experiments and the data is given is appendix 2.69 and 2.70.

(c). For the procedure carried out in basic solution, again reduction of Cr(VI) occurred initially and oxidation and reduction occurred periodically over time. Figure 2.79 and 2.80 represent the absorbance measurements obtained for these experiments and the data is given in appendix 2.71 and 2.72.

Again the reduction of Cr(VI) in acid solution here and the non reduction of Cr(VI) in neutral and basic solution is due to the different oxidising powers of the various Cr(VI) species at different pH values.

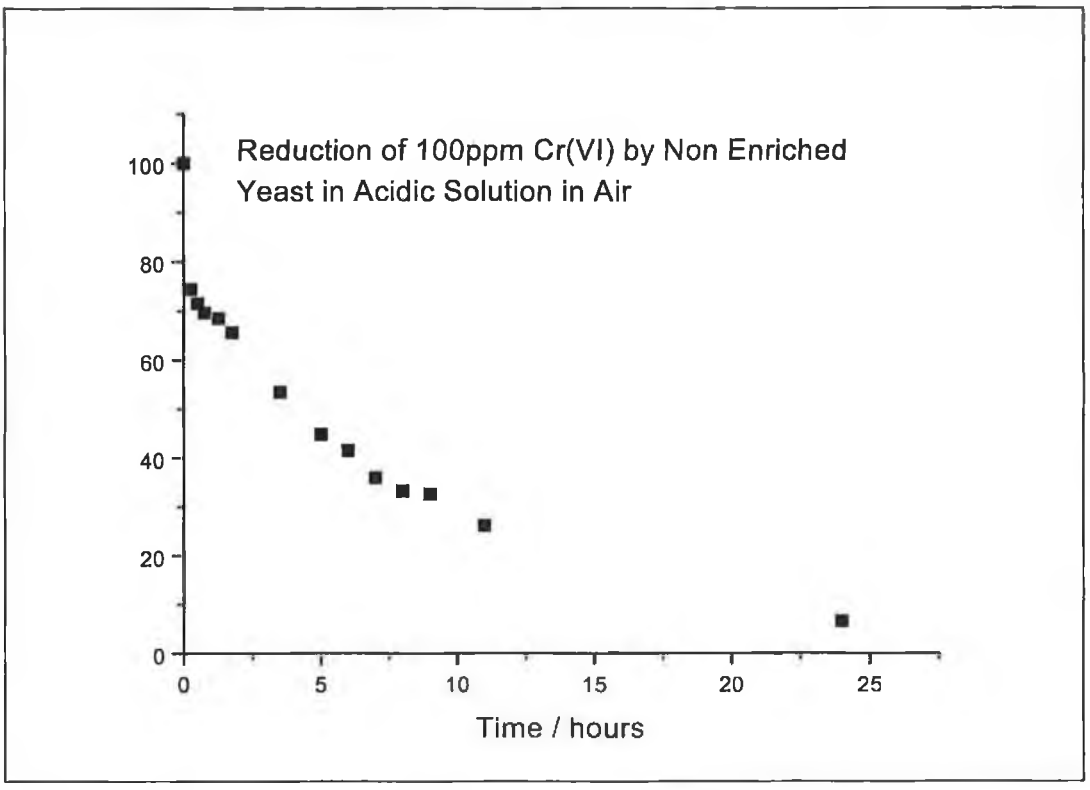


Figure 2.75. Reduction of 100ppm Cr(VI) by non enriched yeast in acidic solution in air.

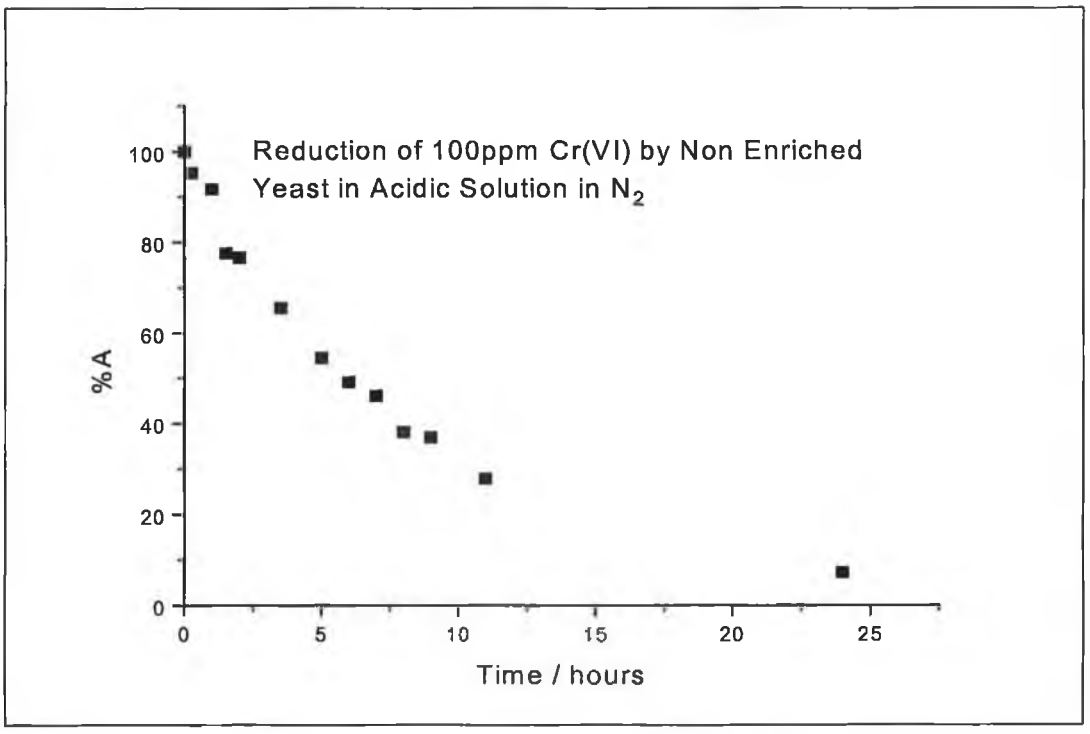


Figure 2.76. Reduction of 100ppm Cr(VI) by non enriched yeast in acidic solution in nitrogen.

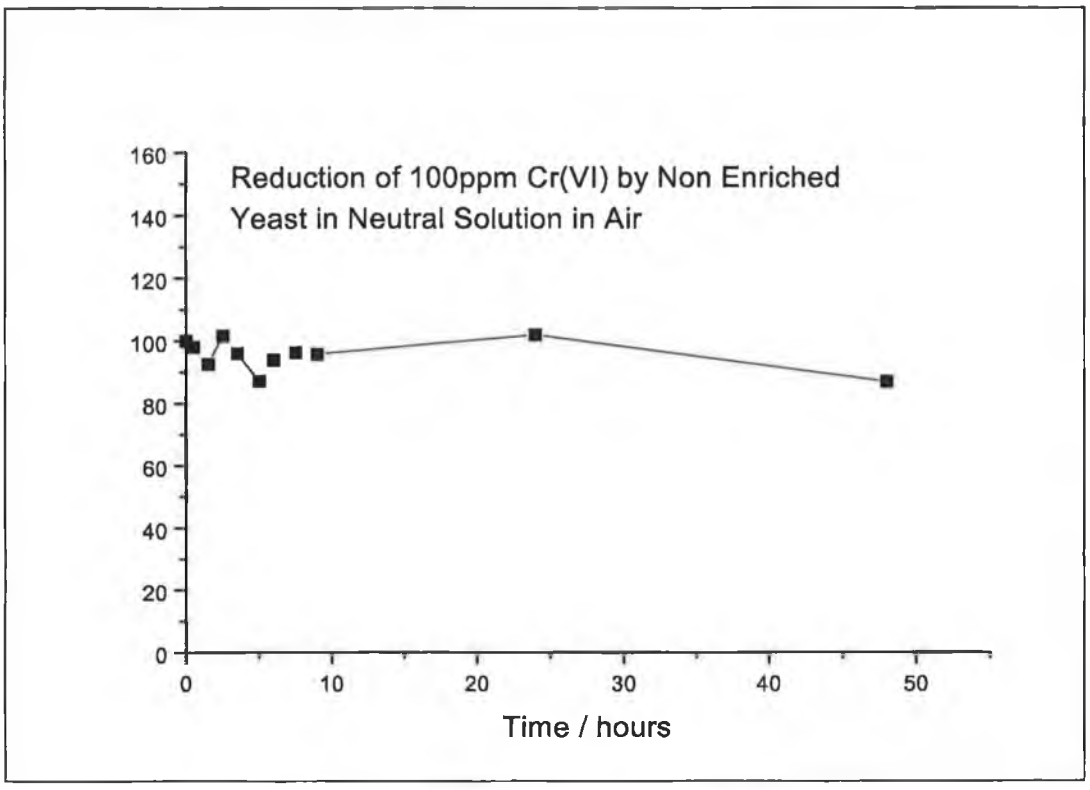


Figure 2.77. Reduction of 100ppm Cr(VI) by non enriched yeast in neutral solution in air.

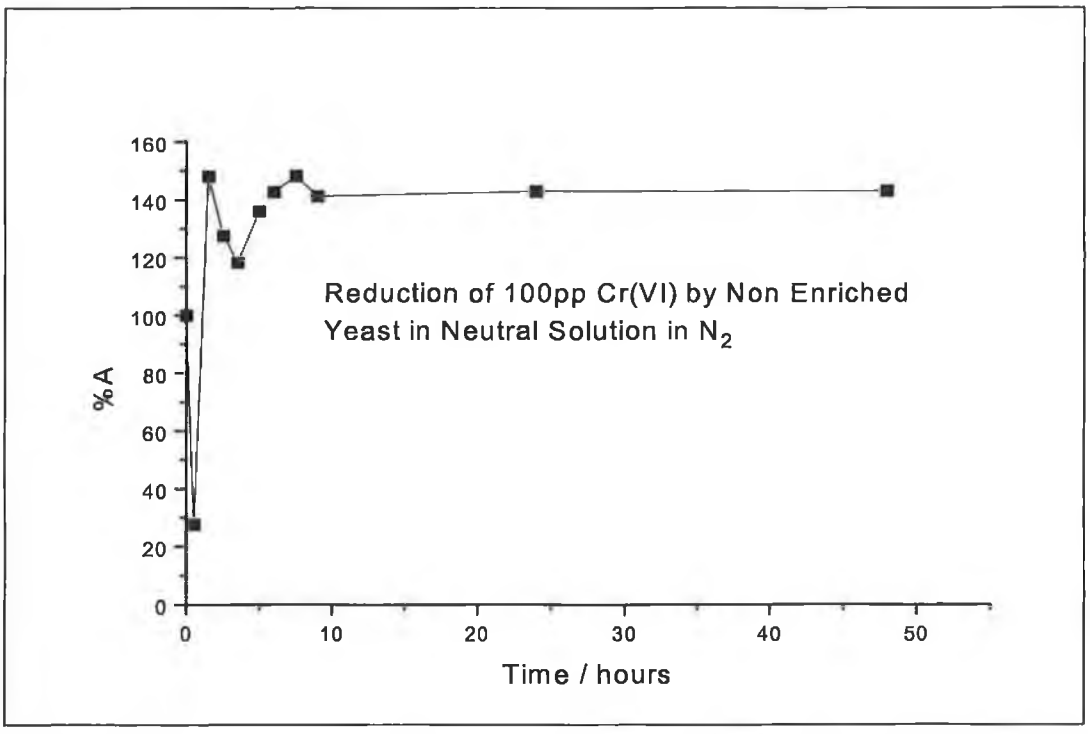


Figure 2.78. Reduction of 100ppm Cr(VI) by non enriched yeast in neutral solution in nitrogen.

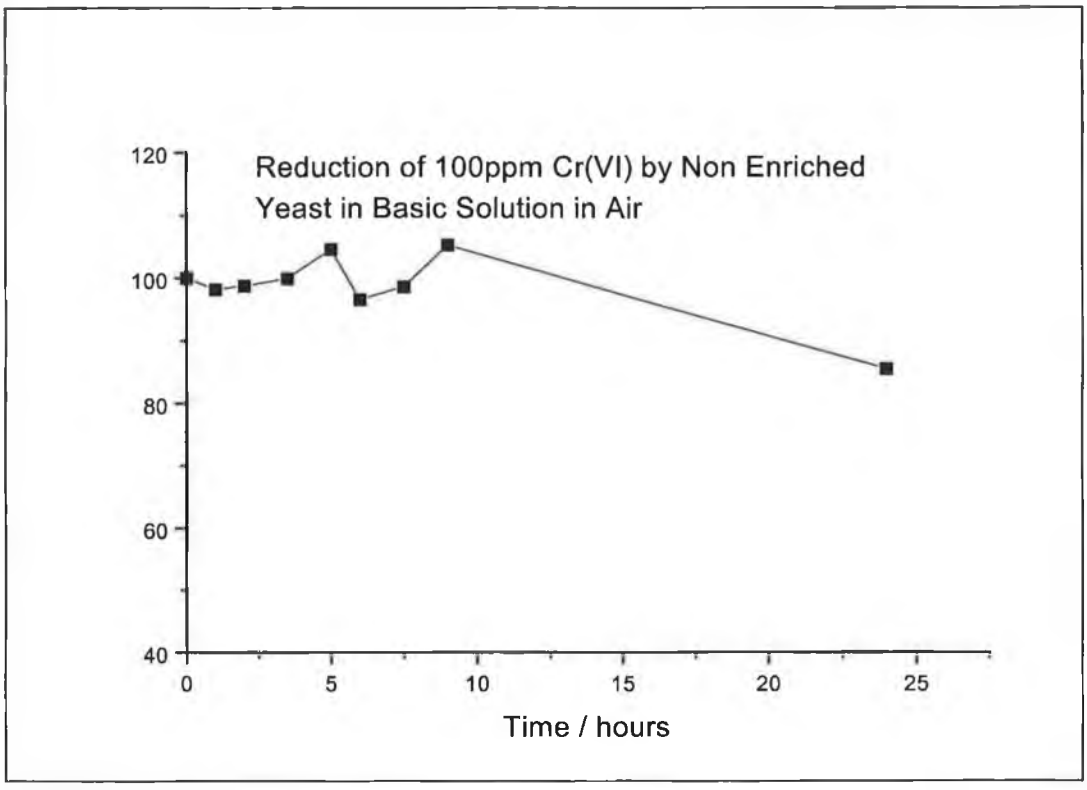


Figure 2.79. Reduction of 100ppm Cr(VI) by non enriched yeast in basic solution in air.

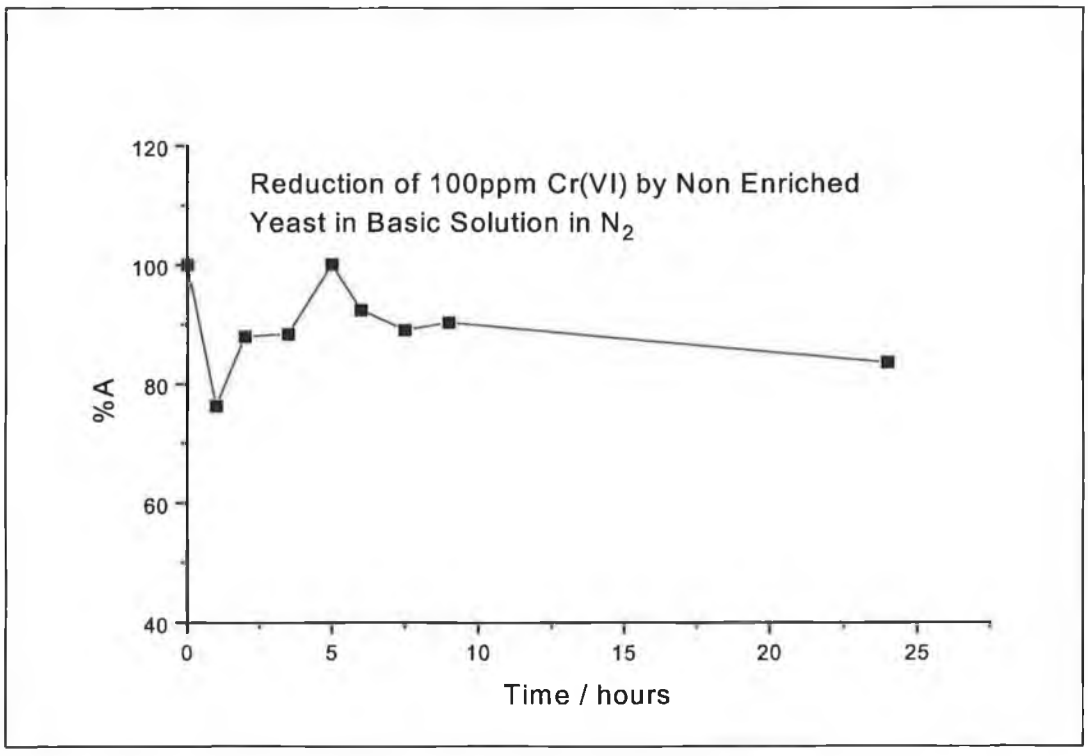


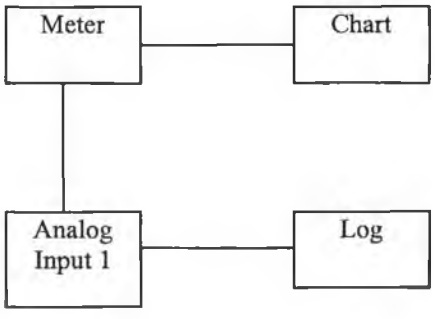
Figure 2.80. Reduction of 100ppm Cr(VI) by non enriched yeast in basic solution in nitrogen.

2.5.2.(d). Kinetic Study of the Rate of Reduction of Cr(VI) by Chromium Enriched Yeast using Redox Potential Measurement.

These experiments were carried out by the addition of chromium enriched yeast to a solution containing H_2SO_4 and $K_2Cr_2O_7$. A redox electrode system consisting of a Pt wire electrode and a double junction Ag/AgCl electrode was placed in the stirred solution. The redox potential (E/mV) was displayed on an Orion EA920 meter. The recorder output of the meter was connected to a Strawberry Tree STI PCjr data acquisition card and the redox potential was read into a computer using Strawberry Tree Quicklog software. The software is operated as follows:

At the DOS prompt type C:\QL\GO.C:\QL

Click the right hand mouse button and select FILE \ OPEN and open the file named Test. This is a simple data acquisition configuration consisting of four icons as shown below and an on-screen chart.



Double-click on the Analog icon and select channel 1. The units are set to Volts\Auto V, resolution is set to 0.025% (1ms), and the sample rate is set to 10 Hertz. Double –click on the meter icon and set the Output type to Fixed Point and the Number Format to 6.3. Double-click on the Log icon and set the sample rate to 1 reading every 10 seconds. Click on the chart icon and set the Time axis to 50 sec and the Y-axis range from 0 to 1 Volt. The file may be saved under a new name.

Recording is started by clicking File\Log\Start All Logs using the right mouse button. recording is stopped by clicking File\Log\Stop All Logs.

In the experiments described here the recording was started on addition of 1g of chromium enriched yeast to the solution of acidic Cr(VI).

1. Redox Potentials of Solutions containing Chromium Enriched Yeast.

The solutions to be analysed were prepared by making a $10cm^3$ aliquot of 1000ppm Cr(VI) up to $100cm^3$ with the chosen buffer as outlined in Table 2.20. After the redox potential of this solution was recorded 1g of the chromium enriched yeast was added (exact quantities are listed in Table2.20) and the redox potentials were recorded over time using the parameters set out above.

Table 2.20. Quantities of $K_2Cr_2O_7$ and chromium enriched yeast added to appropriate buffer.

Volume of 1000ppm Cr(VI) ml	Buffer	Mass of Enriched Yeast g
(i). 10	1M H_2SO_4	1.0002
(ii). 10	0.1M H_2SO_4	0.9994
(iii). 10	0.01M H_2SO_4	0.9998
(iv). 10	0.1M Tris NaOH pH 13	1.0005

It was found that for (i), (ii), and (iii) above the redox potentials decreased to a certain potential after the addition of the chromium enriched yeast to the solution, implying that the chromium enriched yeast initially reduced a certain amount of the Cr(VI) in the solution and that the rate of reduction of the Cr(VI) by the chromium enriched

yeast (rate of reduction of the redox potential) decreased as the H_2SO_4 concentration decreased. The 1M H_2SO_4 reduced the Cr(VI) in 14 seconds, the 0.1M H_2SO_4 took 30 seconds for reduction, while the 0.01M H_2SO_4 took 50 seconds for reduction to occur. Figures 2.81-2.83 show the decrease in the redox potential of the solution with time for these experiments and the data is given in appendix 2.73-2.75.

For (iv) above (alkaline solution) it was observed that the redox potential initially decreased after the addition of the chromium enriched yeast, implying again that the chromium enriched yeast reduced a certain amount of the Cr(VI) that was present in the solution, but then the redox potential increased again, implying that some of the reduced Cr(VI) was reoxidised. The redox potential remained fairly constant from this point. Figure 2.81 represents the redox potentials obtained here and the data is given in appendix 2.76.

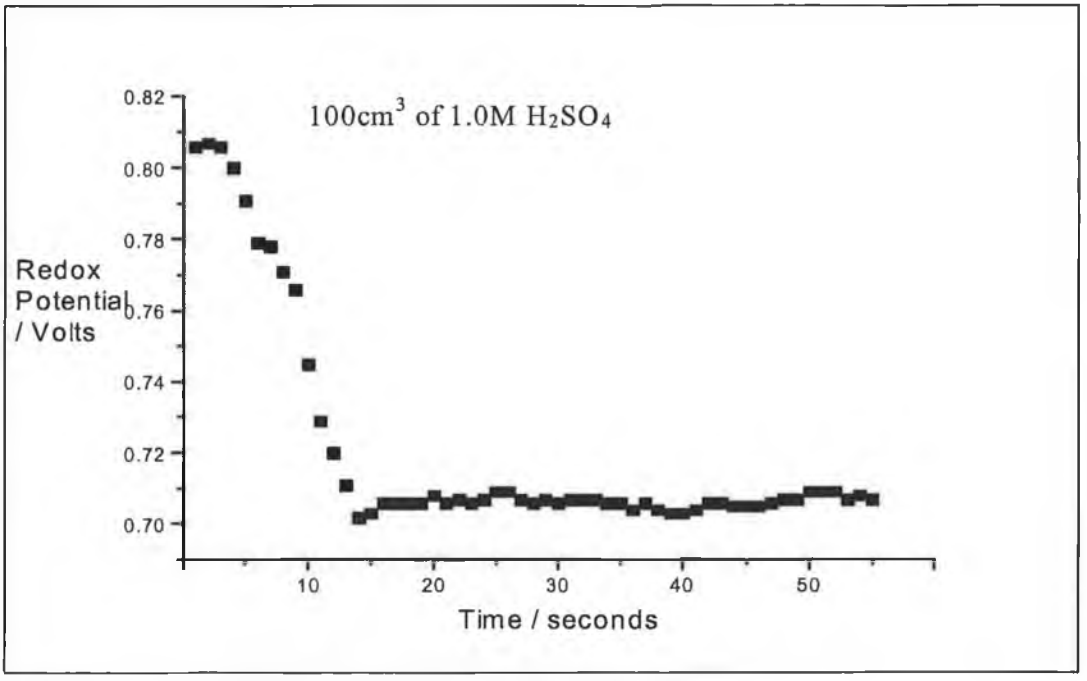


Figure 2.81. Change in redox potential with time on addition of 1g of chromium enriched yeast to 100 cm^3 of 100 ppm Cr(VI) in 1M H_2SO_4.

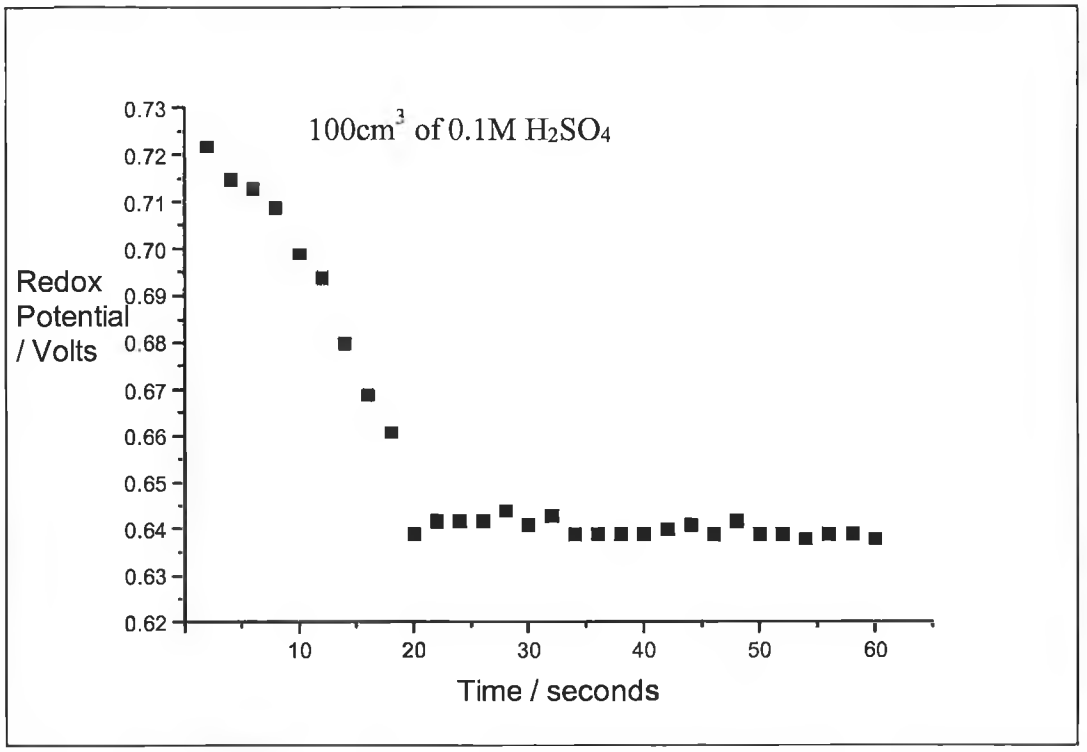


Figure 2.82. Change in redox potential with time on addition of 1g of chromium enriched yeast to 100 cm^3 of 100 ppm Cr(VI) in 0.1M H_2SO_4.

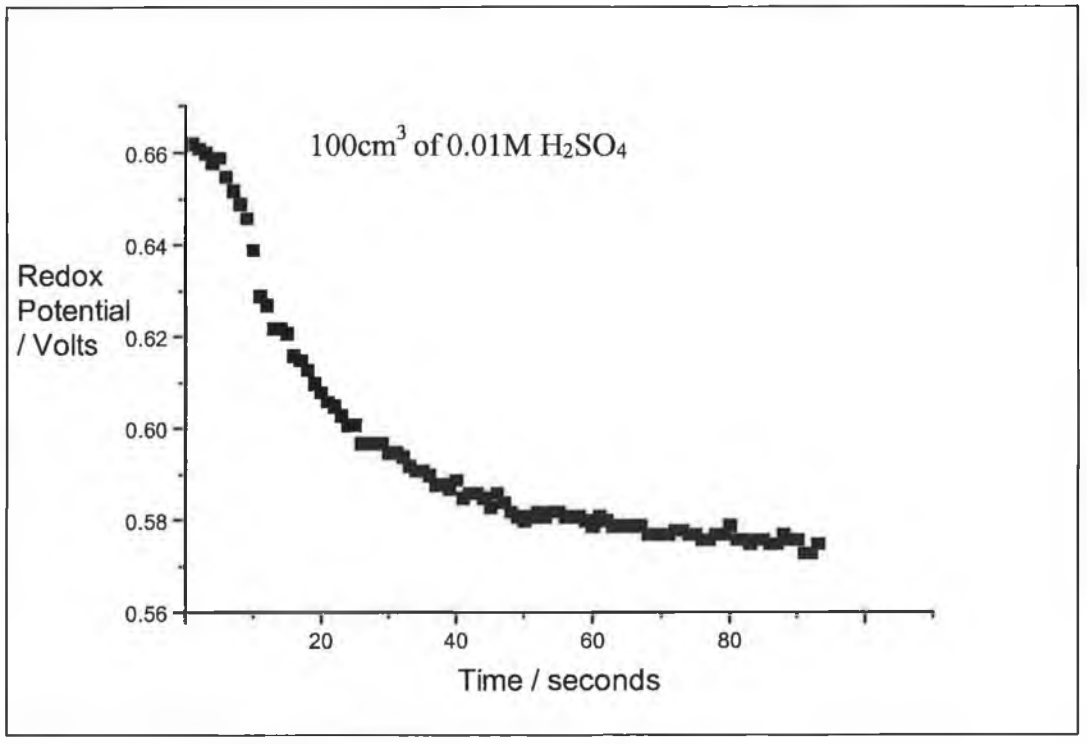


Figure 2.83. Change in redox potential with time on addition of 1g of chromium enriched yeast to 100 cm^3 of 100 ppm Cr(VI) in 0.01M H_2SO_4.

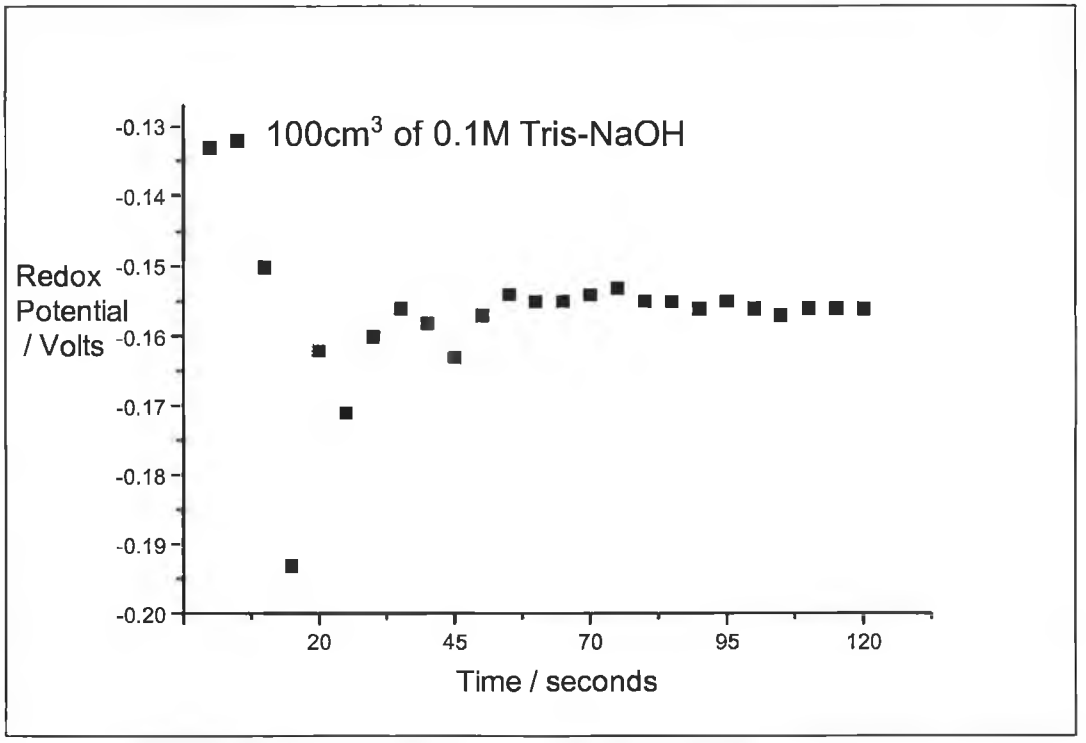


Figure 2.84. Change in redox potential with time on addition of 1g of chromium enriched yeast to 100 cm³ of 100 ppm Cr(VI) in 0.1M Tris-NaOH buffer at pH 13.

2. Redox Titration of Chromium Enriched Yeast using Cr(VI).

These experiments were carried out by the addition of Cr(VI) to a solution containing H_2SO_4 and chromium enriched yeast. A 1g sample of chromium enriched yeast (exact quantities are listed in Table 2.21) was added to $100cm^3$ of 1.0M H_2SO_4 . The redox potential of this solution was recorded using a double junction reference electrode and a platinum wire indicator electrode. A titration was carried out by addition of $0.5cm^3$ aliquots of each of the Cr(VI) standards listed in Table 2.21 to the solution of H_2SO_4 and chromium enriched yeast (except for (vii) below where $1cm^3$ aliquots were added) and the redox potential was recorded after each addition.

Table 2.21. Quantities of chromium enriched yeast and Cr(VI) standard added for titration.

Volume of H_2SO_4 / cm^3	Mass of chromium enriched yeast / g	Cr(VI) / ppm
(i). 100	1.0004	1000
(ii). 100	1.0005	100
(iii). 100	1.0006	10
(iv). 100	1.0002	1
(v). 100	1.0004	0.1
(vi). 100	0.9996	0.01
(vii). 100	-	0.01

For solution (i) to (vi) above it was found that as the Cr(VI) standard was added to the solution of H_2SO_4 and chromium enriched yeast the redox potential of the solution increased and then reached a maximum and eventually levelled off. It was also observed that as the concentration of the Cr(VI) added to the solution decreased the rate at which the redox potential levelled off decreased. Figures 2.85-2.90 represent the titration curves obtained after the addition of the Cr(VI) to chromium enriched yeast and the data is given in appendix 2.77-2.82. Sample (vii) above, which contained no chromium enriched yeast, did not level off even after the addition of $40cm^3$ of 0.01ppm Cr(VI). Figure 2.91 represents the continuous increase in redox potentials obtained in this case after the addition of the Cr(VI) to the H_2SO_4 and the data is given in appendix 2.83

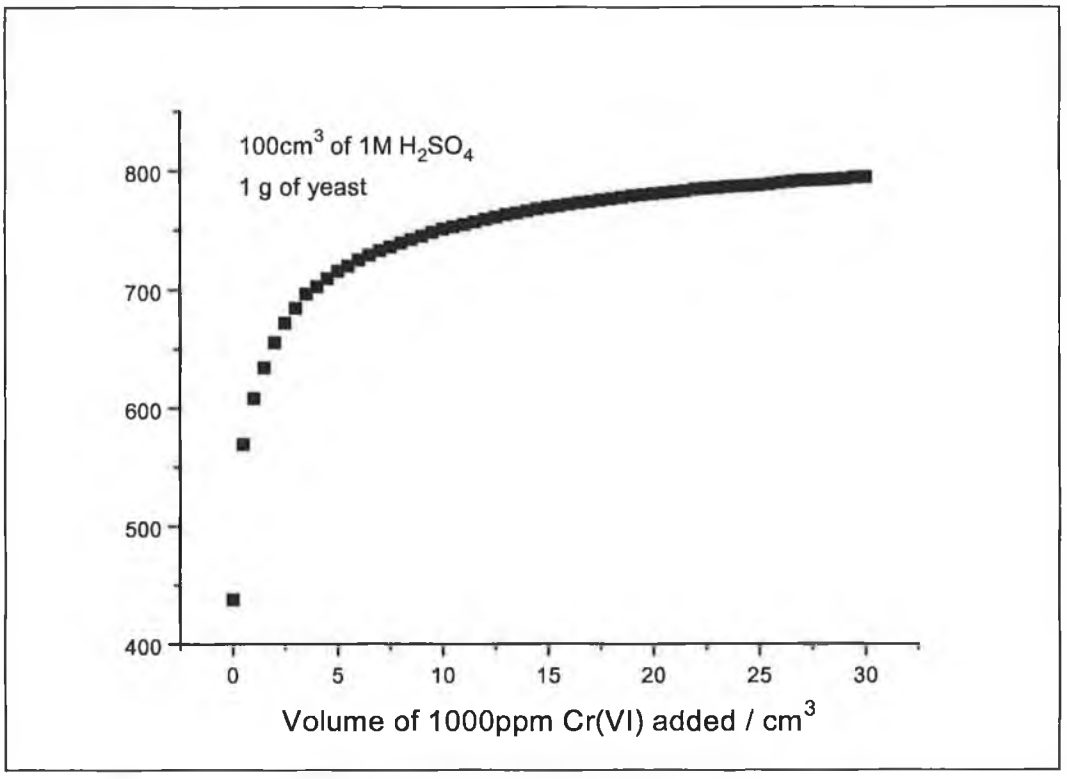


Figure 2.85. Redox potentials after addition of 1000ppm Cr(VI).

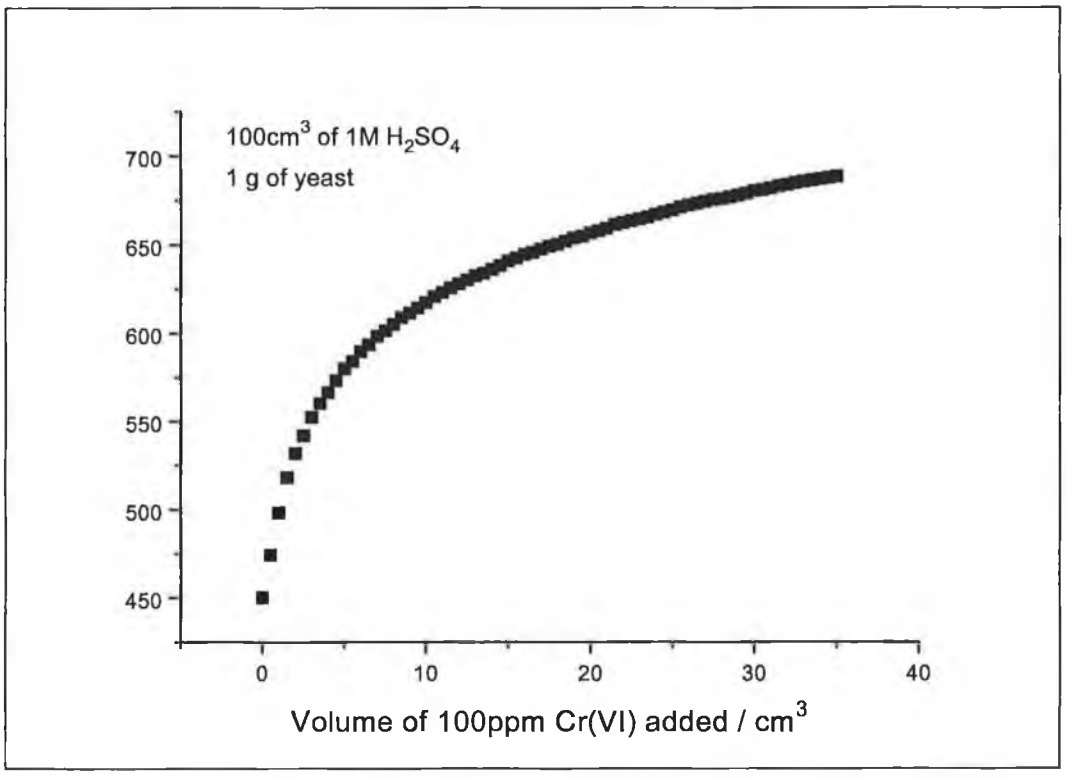


Figure 2.86. Redox potentials after addition of 100ppm Cr(VI).

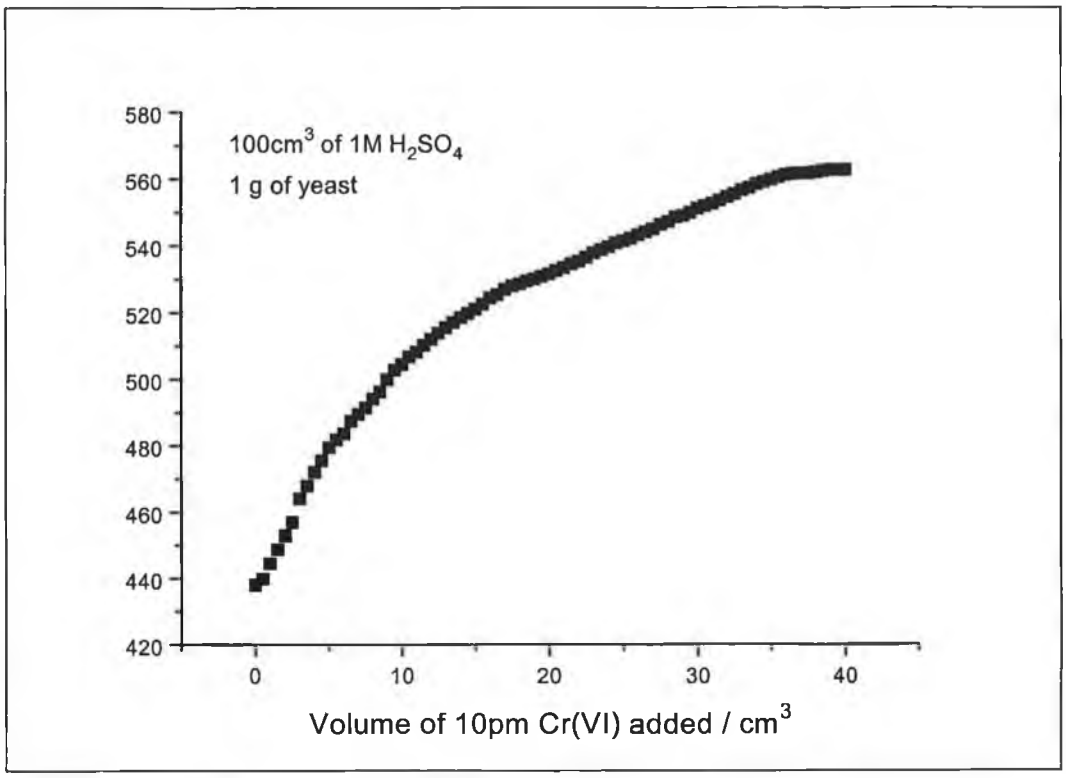


Figure 2.87. Redox potentials after addition of 10ppm Cr(VI).

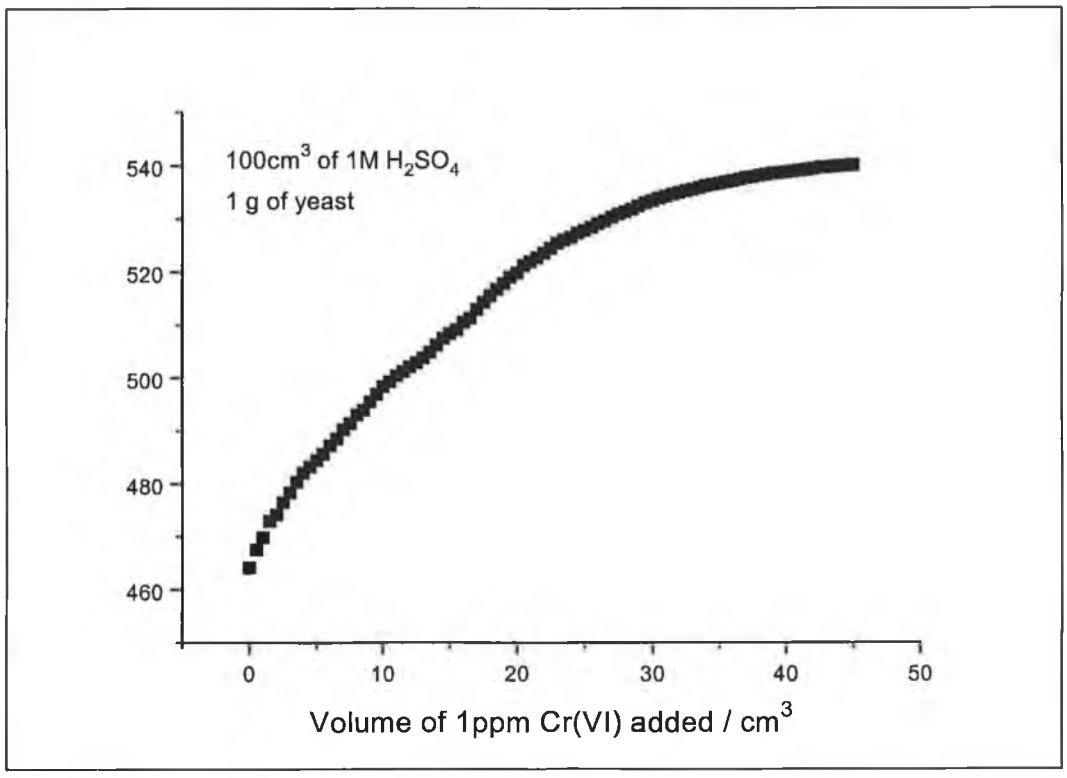


Figure 2.88. Redox potentials after addition of 1ppm Cr(VI).

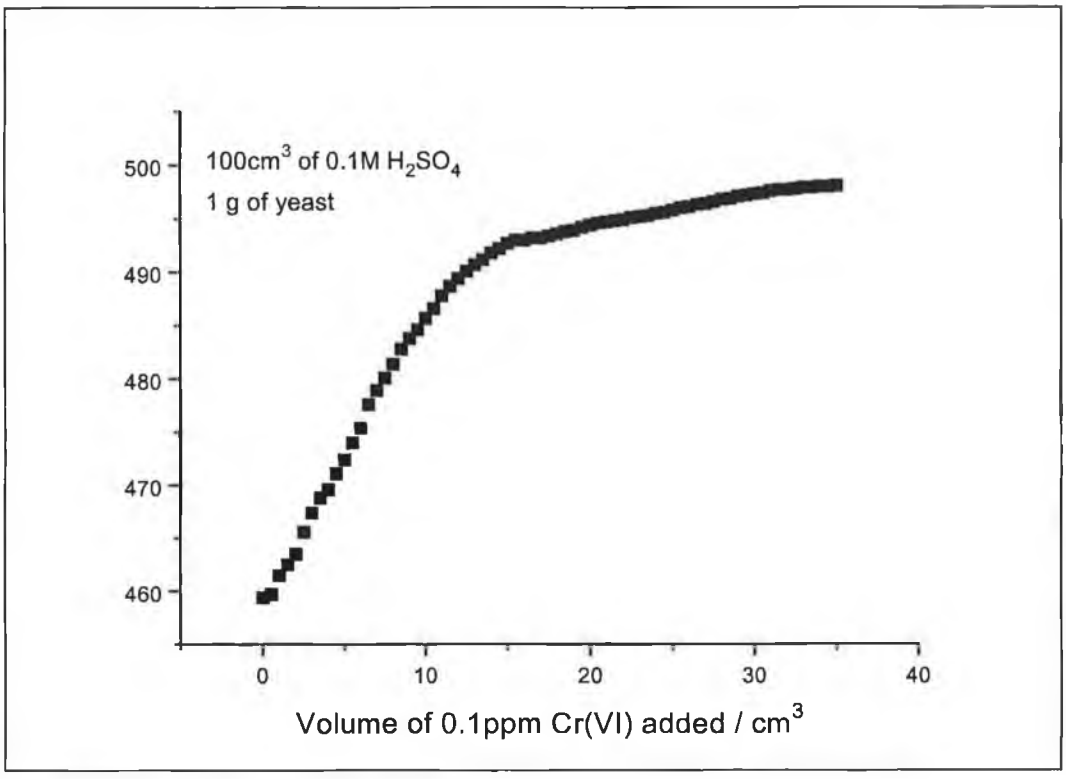


Figure 2.89. Redox potentials after addition of 0.1ppm Cr(VI).

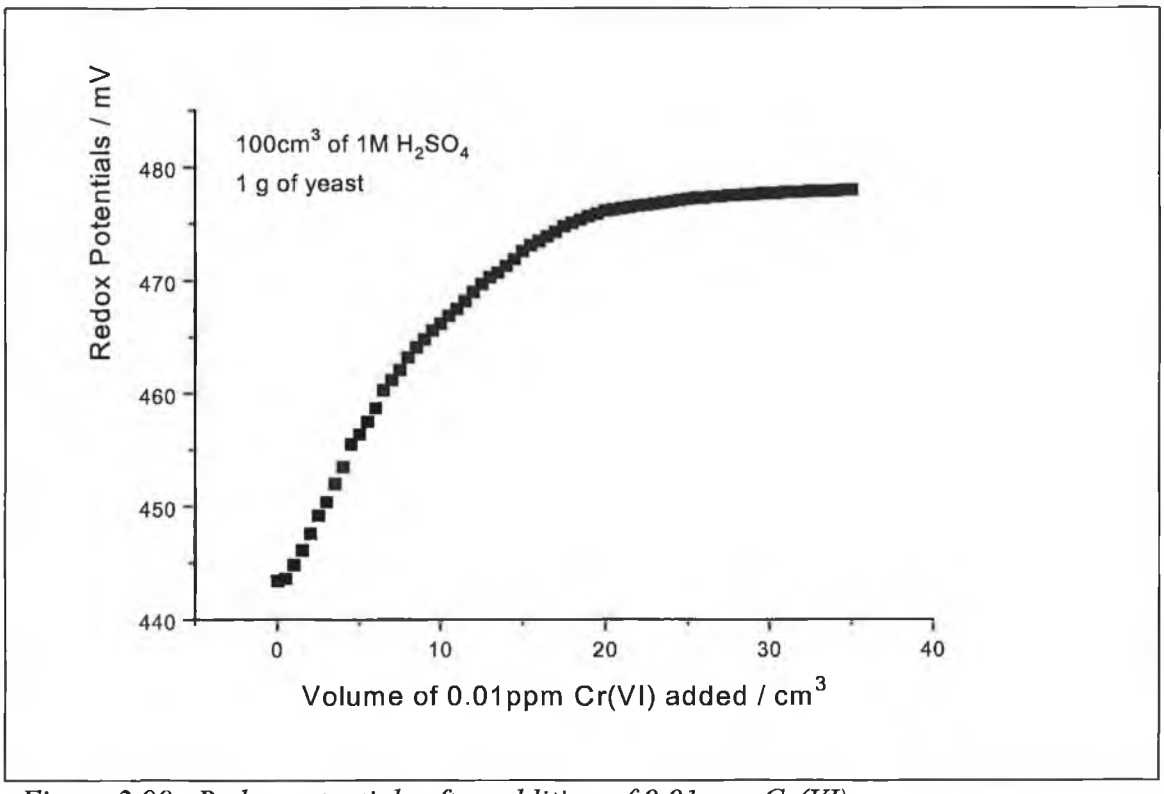


Figure 2.90. Redox potentials after addition of 0.01ppm Cr(VI).

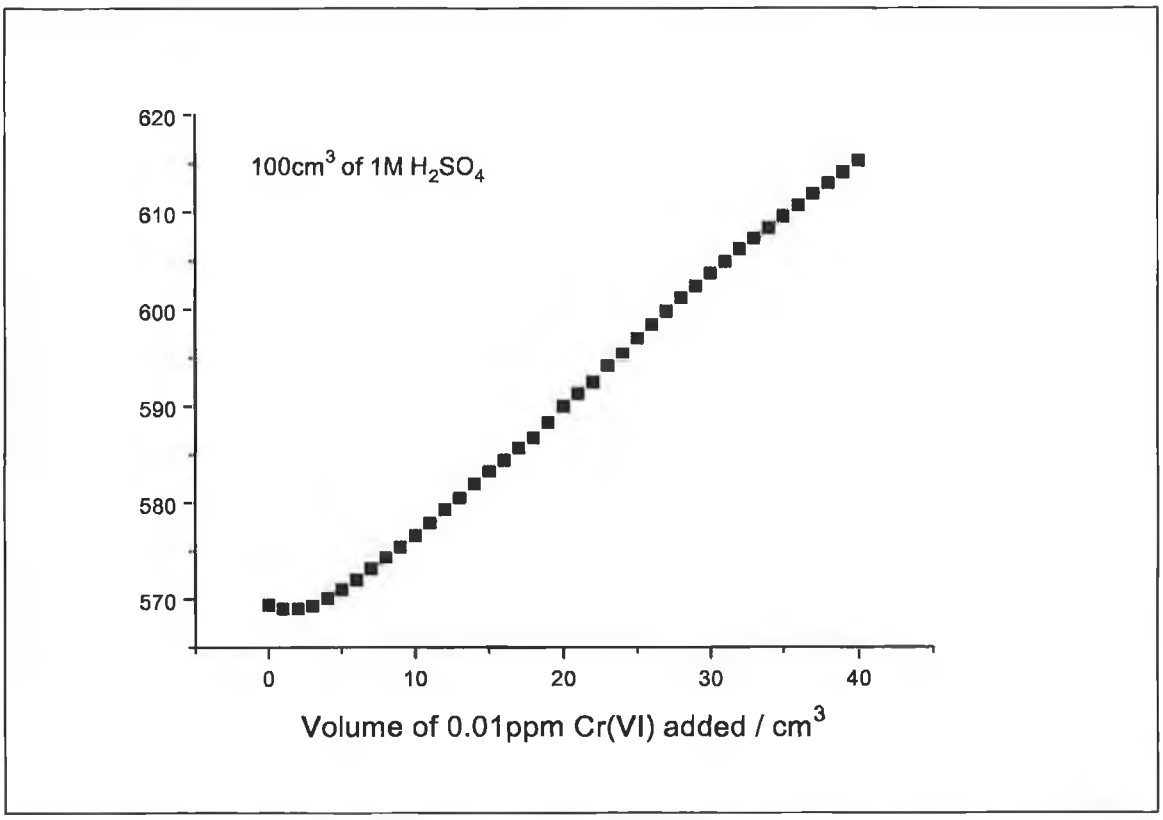


Figure 2.91. Redox potential after addition of 0.01ppm Cr(VI) without addition of chromium enriched yeast.

3.0. Discussion.

3.1. Voltammetry.

Wang (1997) used adsorptive catalytic stripping voltammetry and a phosphate buffer at pH 6.0 containing cupferron as supporting electrolyte for the determination of Cr(VI). Using this method it was found that by applying an initial potential of -100mV (v Ag / AgCl) Cr(VI) was adsorbed onto the surface of a static mercury drop electrode as the Cr(III)-cupferron complex. Then by scanning in a negative direction a chromium reduction peak at -1.0V was observed due to the reduction of the metal centre in the complex.

In the work reported here, the supporting electrolyte contained a peak at -1.0V. This peak was not reported in the original method (Wang (1997)). Attempted removal of this peak was carried using solvent extraction (p 100). This procedure succeeded in reducing the peak in the supporting electrolyte but it failed to totally eliminate the peak (fig 2.3, p 100). It was then suspected that zinc may be the cause of this peak and this was confirmed by addition of 100ppb Zn(II) to the supporting electrolyte (fig 2.4, p 102). The peak could be eliminated by addition of 100μM EDTA to the supporting electrolyte (fig 2.4, p 102). A supporting electrolyte containing 100μM EDTA was then used for further analysis. Figure 2.1 (p 97) represents a typical polarogram obtained for 2ppb Cr(VI) using the conditions outline on page 98.

It was shown that repeated scanning of a 3 ppb solution gave no significant loss of chromium (p 103). An average peak height of 99.4966 +/- 0.8828 was obtained.

A non linear calibration curve was produced from 0 to 5 ppb Cr(VI) with a limit of detection of 0.1ppb (fig 2.7, p 107) which makes this polarographic method suitable for the detection of trace concentrations of Cr(VI). The method may be used for direct

determination of Cr(VI) but due to the curvature of the line it cannot be used for standard additions.

Wang reported that the method also detects Cr(III). A polarogram of 3ppb Cr(III) was obtained (fig 2.5, p105). In a situation where Cr(III) is present in excess, it would be necessary to separate the Cr(III) from the Cr(VI) prior to determination of Cr(VI). It was felt that separation of Cr(VI) from Cr(III) might be possible using an anion exchange column. However it was shown that the chromium in a solution of 1 ppm Cr(III) does not pass through an anion exchange column (fig 2.5, p 105). This experiment was unsuccessful as most of the Cr(III) was lost on the anion exchange column.

Therefore this polarographic method is unsuitable for the analysis of the chromium enriched yeast extract since the excess Cr(III) present in the sample would interfere with Cr(VI) detection. Also the extracting solution is alkaline and so may affect the pH of the supporting electrolyte buffer if a large quantity is used.

3.2. Graphite Furnace Atomic Absorption Spectroscopy.

Using the parameters set out on page 112 a linear calibration curve from 0 to 50 ppb Cr(VI) was produced, giving a limit of detection of 1.36ppb (fig 2.9, p 113). This procedure was then used for the analysis of total chromium in the chromium enriched yeast extract (p 113). It was found that 2800ppm Cr(VI) was present in the chromium enriched yeast extract. This method of detection of chromium is not specific for Cr(VI) and again is unsuitable for the sample under investigation here, unless separation of Cr(VI) and Cr(III) can be achieved.

3.3. U.V. Spectroscopy.

The principle of this method of detection of Cr(VI) is the reaction between chromate and diphenylcarbazide. This reaction is specific for Cr(VI) and the reaction medium must be sufficiently acidic (pH 2) before the reaction will take place. Using the parameters set out on page 117 a linear calibration curve was produced from 0 to 1ppm, in the presence of 8 ppm Cr(III), giving a limit of detection of 43.2 ppb. (fig 2.10, p 119). The Cr(III) was added in order to show that excess Cr(III) will not interfere with Cr(VI) analysis. This would be important when the method would be applied to analysis of yeast extract later. It was found that the stability of the Cr-DPC complex decreased over time. Figure 2.11 (p 120) shows how the colour of the Cr-DPC complex decreased linearly over 5 hours and figure 2.12 (p 121) shows how the colour of the complex decreased exponentially over 6 days and reached 0 on the sixth day. By fitting this graph to the function A*EXP(-B*X) the rate constant for the decay of the colour of the Cr-DPC complex was found to be 0.608 $days^{-1}$. From figure 2.11 (p 120) it can be shown that the absorbance of the Cr-DPC complex decreases by 4.45% in 1 hour. Clearly analysis should be carried out within 30 minutes of the addition of the DPC reagent. This is particularly important in the case of the HPLC method described below (section 3.4.).

3.4. High Performance Liquid Chromatography.

Padarauskas (1998) used on line preconcentration and determination of Cr(VI) by H.P.L.C. with precolumn complexation with 1,5-diphenylcarbazide using a dual column technique. A modified version of this technique, using a single column, and the procedure outlined on page 125 was used for the preconcentration and detection of Cr(VI). Figure 2.13 (p 122) shows the chromatogram obtained for the

preconcentration of 15ppb Cr(VI) using this modified version. Figure 2.14.(a) (p 128) shows 2 large peaks, that of the DPC and the Tris-NaOH pH 13 solutions. A very small peak at 8.74 minutes was also observed. Figure 2.14.(b) (p128) shows the DPC peak, the Tris-NaOH peak and the Cr-DPC peak at 8.6 minutes. The intensities of the DPC and Tris-NaOH peak in figure 2.14.(a) appear to be greater than those in figure 2.14.(b), but this is so because 2.14.(b) was recorded at a lower sensitivity than 2.14.(a). The peak areas in both chromatograms are comparable. The peak at 8.74 minutes in 2.14.(a) would cause negligible error in the measurement of 15ppb Cr(VI). A linear calibration curve from 0 to 25ppb Cr(VI) (fig 2.16, p 131), giving a limit of detection of 6.5ppb was produced using the modified version of the Padarauskas (1998) method. A polarogram of 15ppb Cr(III) was obtained using this method and it was found that no Cr(III) peak appeared in the polarogram. Only the DPC peak and the Tris-NaOH peak were observed.

Since the chromium enriched yeast extract contains excess Cr(III) over Cr(VI) it was investigated to see if Cr(VI) could be detected in excess Cr(III) using this method. A mixture containing Cr(VI) and an equal quantity, a 100 fold excess and a 3300 fold excess of Cr(III) was preconcentrated and eluted (p128). It was found that the Cr(VI) peak, with a similar peak area for each of the mixtures, was observed indicating that Cr(VI) can be detected in the presence of excess Cr(III) and that the presence of Cr(III) does not effect the Cr(VI) peak. Advantages of this preconcentration method using online spectrophotometric detection would include the fact that Cr(III) does not cause an interference and a low limit of detection is obtained.

Of the four methods of detection of Cr(VI) described, voltammetry proved to be the most sensitive, but is unsuitable for the sample under investigation here since the excess Cr(III) present in the chromium enriched yeast extract would cause an

interference. G.F.A.A. spectroscopy has a low limit of detection for chromium, but again is unsuitable for this chromium enriched yeast sample since it is not specific for Cr(VI). U.V. spectroscopy has the advantage of being specific for Cr(VI) but this is not a sensitive method for the detection of Cr(VI). The H.P.L.C. method, again being specific for Cr(VI), is a more sensitive method for the detection of Cr(VI) and it is the method with the best sensitivity that is specific for Cr(VI).

3.5. Extraction of Chromium from Yeast.

1. Choice of Reagent.

Florez-Velez (1995) used Tris-NaOH at pH 13 for the extraction of Cr(VI) from soil samples to which a known quantity of Cr(VI) had been added. They found the percentage recovery of the added Cr(VI) to be 98.6+/-2.2%. This means that this strong alkaline media is efficient for the desorption of chromium in soils. Another advantage of this buffer over an acidic one is that in alkaline solution Cr(VI) is a weak oxidising agent and so will persist longer than it will in a more acidic buffer. Using this Tris-NaOH buffer the extraction process was carried out both in air (p 135) and under nitrogen (p 141) by agitating 1g of yeast with $50cm^3$ of the buffer.

2. Preliminary Experiments on Cr(VI) Extraction.

Initially the importance of air oxidation of Cr(III) to Cr(VI) in alkaline solution was not appreciated. Therefore initial experiments were carried out without any attempt to exclude air. In order to find out if Cr(VI) could be recovered from a yeast sample, a simulated chromium enriched yeast was prepared by addition of 0.0103g of $Cr(III)Cl_3.6H_2O$ to 1g of non enriched yeast. The yeast was extracted using $50cm^3$ of the buffer containing 0, 500 and 1000ppb Cr(VI). After 24 hours the sample was

centrifuged and filtered and diluted to 100 cm^3. The extract now contains 20ppm Cr(III) after dilution.. The extract was analysed daily for Cr(VI) using DPC reagent after acidification of a 5 cm^3 aliquot. The results are given in figure 2.17 (p137). Even where no Cr(VI) was added the hexavalent state was found in the extract after 24 hours of agitation. Where Cr(VI) was added to the extract the hexavalent state was present in higher concentrations after 24 hours. The absorbance indicates that the yeast partially reduces the excess Cr(VI). This is investigated further below. However, over time curves A and B merged while curve C which, contained 1 ppm Cr(VI) at the start was consistently higher. These results indicate that (i) Cr(VI) can be detected in the yeast extract, (ii) yeast partially reduces excess Cr(VI) over a 24 hour period and (iii) the increase in Cr(VI) over time, in the case where no Cr(VI) was added implies air oxidation of Cr(III) to Cr(VI).

It was felt necessary to investigate whether this air oxidation of Cr(III) would still occur even if Cr(III) was bound in the chromium enriched yeast. Therefore two samples were prepared containing (A) 1g of chromium enriched yeast to which free Cr(III) had been added and (B) 1g of the same yeast to which no Cr(III) had been added. Both samples were extracted with 50cm^3 of the Tris-NaOH buffer containing 500ppb Cr(VI). The solutions were centrifuged and filtered and analysed using DPC on a daily basis. The results (fig 2.18, p 139) show the chromium enriched yeast on its own (2.18.B) has partially reduced the added Cr(VI) but the Cr(VI) oxidation eventually occurs over time. The presence of excess free Cr(III) leads to a much higher Cr(VI) level after 24 hours implying easier oxidation of unbound Cr(III) (fig 2.18A).

3. Effect of Air Oxidation.

It was decided to investigate the air oxidation of Cr(III) in the absence of yeast. This was carried out by addition of Cr(III) to the Tris-NaOH buffer, without the addition of any yeast, and the solutions stirred in air and under nitrogen. The extraction in air was carried out by continuously bubbling air through the solution. Figure 2.19A (p 143) shows how the extraction in air produced Cr(VI) after 24 hours of agitation and how the concentration of Cr(VI) increased steadily over 9 days while the extraction under nitrogen (Fig 2.19B, p 143) did not produce Cr(VI) even after 9 days of stirring. This experiment shows how air oxidation of Cr(III) can occur in the presence of air in alkaline solution.

The chromium enriched yeast was then extracted in air (air was not bubbled through) and under nitrogen, centrifuged and filtered after 24 hours, and analysed on a daily basis using DPC. Figure 2.21 (p 145) shows that in the presence of air Cr(VI) was first detected on day 2 and the concentration increased over time, while the extraction carried out under nitrogen produced no Cr(VI) again implying that air oxidation of Cr(III) in the chromium enriched yeast had occurred. This is the first experiment to show that there is no Cr(VI) in the yeast. The limit of detection using the Cr-DPC method (section 2.1.3.(d).) is 43.6ppb. The initial volume of the extract was 100 cm^3. Therefore we can say that the Cr(VI) in the extract is less than 43.6 ppb. The mass of yeast is 1g and therefore we can say that the Cr(VI) in the yeast is less than 21.8 ppm.

4. *Chromium Enriched Yeast Reduces Cr(VI) in Inert Atmosphere.*

To show that the chromium enriched yeast reduces Cr(VI) in an inert atmosphere 500ppb Cr(VI) was added to 50 cm^3 of Tris-NaOH buffer and 1g of the chromium enriched yeast was extracted in air and under nitrogen. The solutions were

centrifuged and filtered after 24 hours, and analysed on a daily basis using DPC. The results are shown in figure 2.20 (p 144). Figure 2.20A shows that Cr(VI) was detected on day 1 for the extraction in air while 2.20B shows no Cr(VI). This experiment is important in that it proved that the chromium enriched yeast will reduce 500 ppb of added Cr(VI) in the absence of air in alkaline solution. In the presence of air Cr(VI) persists in the solution after 24 hours in contact with chromium enriched yeast, and the concentration in the extract increases over time. The rate of oxidation is very slow for 3 days until the reducing capacity of the extract becomes exhausted. Then air oxidation occurs more rapidly. In the absence of air the yeast reduces Cr(VI) and no reoxidation occurs. As Cr(VI) is a weak oxidising agent in basic solution we can expect that the yeast will easily reduce Cr(VI) in more acidic solutions. Also air oxidation of Cr(III) to Cr(VI) will be less likely to occur in acid solution. These conclusions are extremely relevant to the question of Cr(VI) presence in a chromium enriched yeast.

5. Effect of leaving the yeast in contact with the extracting solution.

In previous experiments the bulk yeast was separated from the extract after 24 hours. In the experiments described here the yeast was left in contact with the extract which was sampled on a daily basis and analysed for Cr(VI) as described in section 2.2.3. Figure 2.22.(a) (p 147) shows the visible absorption spectrum of the extract after addition of DPC when 1g of yeast was stirred with 50cm^3 of Tris-NaOH pH 13. The solution was stirred by bubbling air through the mixture and Cr(VI) appears on day 2 and persists for about 4 days before disappearing on day 8. There is no continuous increase in Cr(VI) as happens if the insoluble yeast fraction is separated after 24

hours. The presence of the insoluble fraction appears to provide continuous reduction of air oxidised Cr(VI). In the presence of added Cr(VI), figure 2.22.(b), the added Cr(VI) is barely detectable indicating that the yeast is a reducing agent. Low concentrations of Cr(VI) are detected daily until day 7.

When the chromium enriched yeast was extracted under nitrogen it was expected that no Cr(VI) would be detected. However, Cr(VI) did appear after 24 hours and persists until day 7 (fig 2.23.(a), p 150). Chromium(VI) would not generally appear after 24 hours under nitrogen and its origin in this case is unclear. In the presence of added chromium(VI) under nitrogen the Cr(VI) is barely detectable immediately after addition, indicating reduction of chromium(VI) initially. There is no chromium(VI) even after 24 hours, but it reappears on day 4. Traces of Cr(VI) are detectable each day up to day 7.

These experiments show that yeast reduced Cr(VI). However, over time Cr(VI) seems to appear and this is independent of the absence of air. In both situations the Cr(VI) disappears again. These experiments need to be repeated with greater precautions taken to avoid contamination and to exclude air.

3.6. Preconcentration of Cr(VI) by Ion Exchange.

1. Preconcentration followed by elution with an ammonium buffer and U.V. detection of the Cr-DPC complex.

In the course of this project it became clear that certain techniques would be useful only if (i) the Cr(VI) in solution could be preconcentrated and/or (ii) the Cr(VI) could be separated from the Cr(III).

Cr(VI) may be readily preconcentrated on an anion exchange column. The difficulty was in finding a suitable eluent. The normal eluents for Cr(VI) are based on HCl. Acidic eluents had to be avoided here as Cr(VI) is a strong oxidising agent in acid and

therefore it may be reduced by organic matter on acidification, if the preconcentration procedure was applied to yeast extracts. The eluent arrived at in this work was based on the mobile phase used for ion chromatography of Cr(VI), which is an ammonium sulphate / ammonium hydroxide buffer. A 25cm^3 aliquot of 10ppm Cr(VI) was passed through an ion exchange column and eluted with the buffer and the eluent was collected in 3cm^3 fractions which were analysed for Cr(VI) using DPC.

(i). The Effect of Eluent Concentration and pH.

The effect of the eluent concentration and pH were investigated and it was found that the concentration of the buffer was critical (section 2.3.5.). The results in figure 2.24.(a)-2.25.(b) (p 158-159) indicate that clearly the higher the NH_4OH concentration the faster the Cr(VI) is removed from the column. It was decided to use 2M $(NH_4)_2SO_4$, 4M NaOH, pH 10 because higher concentrations of NH_4OH were too difficult to acidify. It was found that raising the pH to 11.9 (fig 2.26, p 161) gave a much longer elution time. Lowering the pH to 9.0 (fig 2.27. p 161) did not improve the elution time.

(ii). The Effect of Column Length.

The effect of column length was also investigated and it was found that reducing the length of the column significantly reduced the length of time required for the elution of Cr(VI) (fig 2.28, p 162).

(iii). Application to Cr(VI) Standards.

The limit of detection of the diphenylcarbazide method is 43.6ppb. Therefore it was decided to test the ion exchange procedure to see if Cr(VI) could be more readily

detected in eluted fractions than in the original solutions. Before trying out very dilute solutions of Cr(VI) , 25cm^3 aliqouts of 10ppm, 1ppm and 100ppb solutions of Cr(VI) were passed through the column and eluted as described above. The elution profiles are given in figure 2.28-2.30 (p 162-164). None of the solutions would require preconcentration but the quantity of Cr(VI) eluted can not fall too far below that contained in 25cm^3 of 100ppb Cr(VI). Otherwise the maximum absorption of the fractions would be too low. In order to test the preconcentration procedure 1 dm^3 of 5ppb Cr(VI) was passed through the column and eluted. The elution profile is given in figure 2.31(p 164). A purple colour was observed in the fractions. Clearly 5ppb is well below the limit of detection of the spectrophotometric method (43.6ppb) and the method is reliable for the detection of Cr(VI) at this level.

(iv). Percentage Recovery.

We have shown that the preconcentration procedure worked for the detection of Cr(VI) in aqueous samples down to 5ppb. It would only be useful for quantitative analysis if the percentage recovery is 100%. The table of the percentage recovery Table 2.13 (p 166) indicates that the percentage recovery is never 100% and falls to 68% when 25cm^3 of 100ppb Cr(VI) passes through the column. It was not feasible to investigate this further.

(v). Application to Yeast Extracts.

This ion exchange procedure was then applied to the extract when each of the following samples was extracted with 50cm^3 of Tris-NaOH pH 13 (p167).

(i). 1g of chromium enriched yeast

(ii). 1g of non enriched yeast

(iii). 1g of non enriched yeast + 20ppm Cr(III)

All extractions were performed under a nitrogen atmosphere as described in section 2.2.2.(c). The extract was centrifuged, filtered and passed through the column without further dilution. The elution profile for the non enriched yeast samples figure 2.33 (p 168) and figure 2.34 (p 169) show no evidence for Cr(VI). This proves the effectiveness of the procedures for excluding oxygen. The extract for the chromium enriched yeast gave an elution profile typical of Cr(VI) elution (figure 2.32, p 168). It was subsequently shown that if Cr(III) is eluted prior to elution of Cr(VI) the elution profile gives no indication of Cr(VI) in the extract. This is discussed in the next section.

2. Preconcentration followed by elution using ascorbic acid reduction and G.F.A.A. spectroscopic detection.

The principle of this method was the elution of the Cr(VI) using the acidic ascorbic acid to reduce Cr(VI) to Cr(III), which would not be retained by an anion exchange column.

(i). Conditions for the Reduction of Cr(VI).

A 25cm^3 aliquot of 1ppm Cr(VI) was passed through the column and eluted with acidic ascorbic acid (figure 2.35, p 170). The procedure was repeated but the ascorbic acid was allowed to stay in contact with the column for 1 hour to allow the Cr(VI) to be reduced to Cr(III). A visual colour change was observed during this period. All fractions were analysed on G.F.A.A. The elution profile is given in figure 2.36 (p 171). It was found that a more ideal profile was obtained after the ascorbic acid was left in contact with the column for 1 hour prior to elution to allow time for complete reduction of Cr(VI) to Cr(III).

(ii). Application to Cr(VI) Solutions.

Elution of 25cm^3 aliqouts of 1000ppb, 100ppb and 100cm^3 of 10ppb Cr(VI) using the above method gave the elution profiles in figure 2.36-2.38 (p 171-172). In order to test the procedure 1dm^3 of 1ppb Cr(VI) was passed through the column and eluted and the fractions analysed on G.F.A.A.. The elution profile (figure 2.39, p 173) indicates the presence of Cr(VI) in the fractions. The technique could be used to detect Cr(VI) in water samples down to 1ppb Cr(VI).

(iii). Application to Yeast Extracts.

1g of chromium enriched yeast and 1g of non enriched yeast were extracted using 50cm^3 of Tris-NaOH buffer (page 173) and extracts were passed through an ion exchange column. The elution profiles are given in figure 2.40 and 2.41 (p 174). As pointed out in the experimental G.F.A.A. did not appear to work well in this application indicating chromium in the non enriched yeast extract. Clearly this is wrong and this procedure is of no further value.

3.7. Separation of Cr(III) and Cr(VI).

(i). Application to Pure Solutions.

In the procedure above no attempt was made to allow for the possibility that Cr(III) might be absorbed onto the ion exchange column. Cr(III) may form anionic hydroxy complexes which adhere to the column and therefore Cr(III) should be removed from the column using H_2SO_4 prior to elution of the Cr(VI). This would be essential if analysis of the eluent is carried out using a technique such as GFAA which is not specific for Cr(VI).

A 25cm^3 mixture of 50ppm Cr(III) and 100ppb Cr(VI) was passed through the column and the effluent collected (page 175). It was rinsed with distilled water until 50cm^3 had been collected. It was found that only 76.2% of the chromium passed through the column, so Cr(III) was retained. The Cr(III) was eluted with 0.1M H_2SO_4 and the elution profile shows the removal of the Cr(III) (figure 2.42, p 177). Cr(VI) was then eluted with $(NH_4)_2SO_4/NH_4OH$ pH 10 buffer and the elution profile is given in figure 2.43 (p 177). This procedure in quite successful in demonstrating the separation of Cr(III) and Cr(VI).

In order to show that no air oxidation occurs during the process the ion exchange procedure was repeated using 25cm^3 of 100ppm Cr(III) which had been agitated for 24 hours, centrifuged and filtered as described on page 178. It was found that 79% of the chromium passed through the column. The elution profile of the retained Cr(III) is given in figure 2.44 (p 179). There is no evidence for Cr(VI) in the fractions figure 2.45 (p 180).

(ii). Application to the Yeast Extract.

A 25cm^3 aliqout of the chromium enriched yeast extract was passed through the column and it was found that 78% of the Cr(III) in the extract passed through. The elution profile for the Cr(III) is given in figure 2.46 (p 181) and for Cr(VI) in figure 2.47 (p 182). No colour change was observed in the fractions and no evidence for Cr(VI) is observed. This experiment confirms the results from 1.(v) in section 3.6 above. However, the elution profile figure 2.47(p 182) is much better than figure 2.32 (p 168) as a consequence of removing the Cr(III).

However in order for this preconcentration procedure to offer any advantage over direct analysis of the extract, a larger volume of the solution of the extract would have

to be passed through the column as in the preconcentration of 5ppb Cr(VI) as described in page 163.

3.8. Preconcentration by H.P.L.C.

(i). Application to Chromium Enriched Yeast Extract.

The preconcentration procedure discussed in section 3.4 above was applied to the chromium enriched yeast extract (extracted in nitrogen), by adding DPC to $20cm^3$ of the extract, and $12cm^3$ was preconcentrated and eluted from the column. The chromatogram in figure 2.48.(a) (p 185) shows no evidence for the presence of Cr(VI). The detection limit for this H.P.L.C. method is 6.5ppb. Therefore the Cr(VI) concentration in the extract is less then 6.5ppb. Since 1g of yeast was extracted with $50cm^3$ of Tris-NaOH buffer which was not further diluted, the concentration of Cr(VI) in the yeast is less than 0.325ppm. By preconcentrating a larger mass of yeast this detection limit could be significantly lowered.

(ii). Reduction of Trace Cr(VI) by Chromium Enriched Yeast.

13ppb, 44ppb and 88ppb Cr(VI) were added to the chromium enriched yeast extract and preconcentrated and eluted from the column. Figure 2.48 (p 185) shows the resulting chromatograms. For the solution containing 13ppb Cr(VI) a very small Cr(VI) peak was observed and on comparing this peak height to that of a 15ppb Cr(VI) standard it can be seen that the peak area in 2.48.(b) is greatly reduced compared to the standard, implying that the chromium enriched yeast extract reduced the added Cr(VI). Chromatograms 2.48.(b) and 2.48.(c) show the resulting chromatograms after the addition of 44ppb and 88ppb Cr(VI). Again on comparing these peak areas with those of standards it is obvious that the chromium enriched

yeast extract is capable of reducing added Cr(VI). It was not tested over time to see if any further reduction took place.

3.9. Determination of the Rate of Reduction of Cr(VI) by Ascorbic Acid.

The aerobic reduction of Cr(VI) using ascorbic acid was studied at pH 7.4 using Tris-HCl buffer. The reaction was carried out using pseudo first order conditions as described in section 2.5.1.(d) (p 190). The results are shown in figure 2.49-2.54 (p 191-194). A plot of the pseudo first order constants against ascorbic acid concentration gives the rate constant for the reaction (fig 2.55, p 195). The value obtained was 0.366 dm^3 mol^{-1} s^{-1} which compares with 0.56+/-0.02 (Dixon (1993)) and 0.36+/-0.01 (Connett(1985)). The zero intercept and linear plot is consistent with the expression

$$K_{obs} = a[\text{ascorbic acid}]\,[Cr(VI)]$$

The postulated mechanism involves formation of a Cr(VI)-ascorbic acid ester (p 86) in which case the following equation would apply

$$HA + Cr(VI) \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} HACr(VI)$$

$$HACr(VI) \xrightarrow{k_3} Cr(IV) + A$$

Where A = dehydroascorbate. The rate law consistent with this mechanism is

$$\frac{-dCr(VI)}{dt} = \frac{k_3 k_1 [AscorbicAcid][Cr(VI)]}{k_{-1} + k_3}$$

Therefore $$a = \frac{k_3 k_1}{k_{-1} + k_3}$$

If the redox reaction is rapid then $k_3 >> k_{-1}$ and the formation of the ester will be the rate determining step and $a = k_1$.

O'Brien (1996) found that in Tris-HCl buffer the rate constant was oxygen dependent. This oxygen dependence was briefly studied here but little effect was observed Fig 2.56, p 197). The oxygen dependence may be due to reaction between Cr(VI) and the Tris-HCl buffer. No oxygen dependence was found in phosphate or HEPES buffers.

3.10. Reduction Capacity of Chromium Enriched Yeast.

The purpose of these experiments was to determine if the chromium enriched yeast is a reducing agent. Various concentrations of Cr(VI) were allowed to contact 0.25g of (i) chromium enriched yeast and (ii) non enriched yeast, under acidic, neutral and basic conditions, both in the presence of air and absence of air. The Cr(VI) concentration of the supernate was measured over time.

(i). Reduction of Cr(VI) by chromium enriched yeast in acid solution.

Figure 2.57-2.62 (p 202-205) compare the reduction of 200, 100 and 50ppm Cr(VI) by chromium enriched yeast in 1M H_2SO_4 in air and under nitrogen. Cr(VI) is readily reduced in all cases. The curves do not obey any simple rate law. The presence or absence of air appears to have no effect on the rate of reduction. The concentration of Cr(VI) appears to have no effect on the rate of reduction. For example the absorbance of the Cr(VI) falls to about 10% of its original value after 24 hours in all cases (measured under nitrogen). It is therefore unlikely that Cr(VI) can exist in the presence of chromium enriched yeast in acid solution.

The experiment was repeated using non enriched yeast under air and nitrogen and very similar results were obtained in each case (figure 2.75-2.76, p 215). However the final Cr(VI) concentration had fallen to 5% of its original value in 24 hours.

(ii). Reduction of Cr(VI) by chromium enriched yeast in neutral solution.

Figure 2.63-2.68 (p 206-208) shows the reduction of 200, 100 and 25ppm Cr(VI) in neutral (pH7.4) solution in air and under nitrogen. At this pH the behaviour of the reduction process in very dependant on Cr(VI) concentration. At the highest concentration (200ppm) the Cr(VI) absorbance never falls below approximately 80%. In air the %A oscillates before increasing dramatically after 24 hours. This could be explained by air oxidation of the Cr(III) in the enriched yeast. Under nitrogen this dramatic increase does not occur, confirming the oxidation hypothesis. However, under nitrogen oscillation does occur. It is difficult to explain how oxidation of Cr(III) could occur under these conditions. It is possible that other absorbing species are formed which are interfering with Cr(VI) measurement.

Using 100ppm Cr(VI) the %A falls to 65% and the oscillation of the %A absorbance is clearly defined. Similar behaviour occurs under nitrogen (fig 2.65-2.66, p 207). On reducing the Cr(VI) concentration to 25ppm very little change in absorbance occurred in air or in nitrogen (fig 2.67-2.68, p 208). When non enriched yeast was used a rapid reduction of the %A occurred initially in nitrogen. This was followed by a dramatic increase. Very little change occurred in air (fig 2.77-2.78, p 216).

(iii). The Reduction of Cr(VI) by Chromium Enriched Yeast in Basic Solution.

Figure 2.69-2.74 (p 210-212) shows the reduction of 200, 100 and 25ppm Cr(VI) in (basic pH 13) solution, in air and under nitrogen. For the 200ppm solution oscillation of the %A is observed followed by increasing oxidation of Cr(III) to Cr(VI) in air but not in nitrogen. For the 100ppm solution oscillation is observed, followed by increase in oxidation in both cases. For the 25ppm solution air oxidation is much more

dramatic than under nitrogen. In the case of non enriched yeast (fig 2.79-2.80, p 217) there is much less variation in the %A in both cases.

The following conclusions and observations may be drawn,

(i). Only in acid solution is Cr(VI) reduced effectively by either chromium enriched yeast or non enriched yeast.

(ii). In neutral and basic solutions the %A oscillates.

3.11. Kinetic Study of the Rate of Reduction of Cr(VI) by Chromium Enriched Yeast using Redox Potential Measurements.

Figure 2.81-2.83 (p 220-221) shows the change in potential at a platinum wire placed in 100ppm Cr(VI) on addition of 1g of chromium enriched yeast. The drop in potential means that the added reagent is reducing. The time taken for the equilibrium potential to be reached depends on H_2SO_4 and in faster in 1M H_2SO_4. This indicates very rapid reduction of Cr(VI) in acid solution. In base (fig 2.84, p 222) the redox potential oscillates. This in reminiscent of the absorbance of Cr(VI) in neutral and basic solution. However, the net change in the redox potential was much smaller than in acid solution.

When the experiments were performed by addition of Cr(VI) to 1g of chromium enriched yeast in 1M H_2SO_4 (fig 2.85-2.90, p 224-226), continuous increase in the redox potential was observed until a certain potential was reached then the redox potential began to level off implying again the chromium enriched yeast was capable of reducing the added Cr(VI).

Conclusion.

(a). In this thesis four methods for the determination of Cr(VI) have been investigated. It has been shown that

(i). the spectrophotometric method using DPC is specific for Cr(VI) in the presence of excess Cr(III).

(ii). preconcentration of Cr(VI) on a C_{18} column as a Cr-DPC complex is specific for Cr(VI) in the presence of excess Cr(III).

(iii). the limit of detection of the H.P.L.C. method (6.5ppb) is superior to the spectrophotometric method (43.2ppb).

(b). Two ion exchange procedures have been developed for the preconcentration of Cr(VI). It has been shown that

(i). Cr(VI) at a level of 5ppb can be detected by elution with an ammonia buffer and spectrophotometric detection.

(ii). Cr(VI) at a level of 1ppb can be detected by elution with acidic ascorbic acid and G.F.A.A. detection.

(c). A procedure has been developed for the separation of trace Cr(VI) from excess Cr(III) by ion exchange.

(d). The importance of air oxidation in Cr(VI) speciation has been discussed. It has been shown that

(i). Cr(III) is oxidised by air in alkaline solution in the timescale typically used for Cr(VI) extraction.

(ii). air oxidation of Cr(III) increases with time.

(e). The extraction of Cr(VI) from a chromium enriched yeast has been studied. It has been shown

(i). that in air Cr(III) in the extract is oxidised to Cr(VI) after 24hours.

(ii). that there is no Cr(VI) in the extract after 24 hours if the procedure is carried out under nitrogen as evidenced by spectrophotometric and H.P.L.C. analysis of the extract.

(f). The reduction of Cr(VI) by yeast samples in acidic, neutral and basic solution has been studied. It is shown

(i). that yeast samples effectively reduce a large excess of Cr(VI) in 1M H_2SO_4.

(ii). that in neutral and basic solution the reduction is often reversible, both in air and under nitrogen.

(g). The rate of reduction of Cr(VI) using a model compund, ascorbic acid was determined at pH 7.4 and the results compared with previous studies.

(h). Further studies need to be carried out in the following areas,

(i). the H.P.L.C. method and the ion exchange method could be applied to larger volumes of extract.

(ii). the reduction of Cr(VI) by yeast at different pH values.

Appendices.

2.1. Effect of Initial Potential on the Cr(VI) Peak Height.

E Initial / mV	Peak Hieght / nA
-650	54.700
-700	51.300
-750	47.150
-800	40.800
-850	34.850
-900	31.150
-950	28.200

2.2. Effect of Varying Drop Growth Time on Cr(VI) Peak Height.

Drop Size	Peak Height / nA
0.1	25.666
0.2	30.333
0.3	36.184
0.4	42.841
0.5	48.666
1.0	67.215
1.5	85.992
2.0	102.666
2.5	125.333

2.3. Effect of Multiple Scanning on Cr(VI) Peak Height.

Scan Number	Peak Height / nA
1	100.333
2	99.800
3	100.450
4	98.500
5	98.400

2.4. Effect of Electrolysis Time on Cr(VI) Peak Height.

Electrolysis Time	Peak Height / nA
0	41.600
10	87.800
20	168.000
30	228.000
40	248.500
50	240.500
60	239.500

2.5. Calibration Curve for Polarography

ppb Cr(VI)	Peak Height / nA	Plotted / nA
0	12.70	0.00
1	40.00	27.30
2	63.30	50.60
3	81.70	69.00
4	94.15	81.45
5	104.00	91.30

2.6. Calibration Curve for G.F.A.A. Spectroscopy.

ppb Cr(VI)	Absorbance
0	0.000
10	0.041
20	0.077
30	0.115
40	0.154
50	0.196

2.7. Calibration Curve for U.V. Spectroscopy.

ppm Cr(VI)	Absorbance
100	0.071
200	0.117
300	0.172
400	0.216
500	0.268
600	0.314
700	0.358
800	0.412
900	0.467
1000	0.504

2.8. Effect of Time on Absorbance (hours).

Time / hours	Absorbance
0	0.289
1	0.276
2	0.265
3	0.247
4	0.237
5	0.226

2.9. Effect of Time on Absorbance (days).

Time / days	Absorbance
0	0.267
1	0.151
2	0.080
3	0.040
4	-
5	-
6	0.000

2.10. Calibration Curve for H.P.L.C.

ppb Cr(VI)	Peak Height / nA x 10^{-5}
0	0
5	2.09 x 10^{-5}
10	4.99 x 10^{-5}
15	9.11 x 10^{-5}
20	12.78 x 10^{-5}
25	

2.11. To Show That Cr(VI) Can Be Extracted From The Yeast.

Time / days	Non-Enriched Yeast + 20ppm Cr(III) / Absorbance	Non-Enriched Yeast + 20ppm Cr(III) + 500ppb Cr(VI) / Absorbance	Non-Enriched Yeast + 20ppm Cr(III) + 1ppm Cr(VI) / Absorbance
1	0.035	0.088	0.098
2	0.037	-	0.107
3	0.048	-	0.114
4	-	0.096	0.122
5	-	0.110	-
6	0.106	0.133	-
7	0.120	0.136	0.181
8	0.140	0.149	0.231
9	0.168	0.177	0.263

2.12.To Show that Cr(VI) can be Extracted in Presence of Chromium Enriched Yeast.

Time / days	Yeast + 20 ppm Cr(III) + 500 ppb Cr(VI)	Yeast + 500 ppb Cr(VI)
1	0.164	0.084
2	-	0.096
3	-	0.094
4	0.236	0.134
5	0.232	-
6	0.239	0.170
7	0.257	0.174
8	0.271	0.202
9	0.316	0.207

2.13.To Show Air Oxidation Of Cr(III) To Cr(VI) In Alkaline Solution.

Time / days	Tris NaOH + 20 ppmCr(III) in Air	Tris-NaoH + 20 ppm Cr(III) in Nitrogen
0	0	0.00
1	0.045	0.00
2	0.087	0.00
3	-	0.00
4	-	0.00
5	0.154	0.00
6	0.179	0.00
7	0.193	0.00
8	0.233	0.00
9	0.263	0.00

2.14. To Show that Chromium Enriched Yeast Reduces Cr(VI) In Inert Atmosphere.

Time / days	Yeast + 500 ppb Cr(VI) in Air	Yeast + Cr(VI) in Nitrogen
1	0.084	0.00
2	0.096	0.00
3	0.094	0.00
4	0.134	-
5	-	0.00
6	0.170	0.00
7	0.174	0.00
8	0.202	0.00
9	0.207	0.00

2.15. To Show Air Oxidation Of The Chromium Enriched Yeast Extract.

Time / days	Yeast in air	Yeast in Nitrogen
1	0.000	0.000
2	0.142	0.000
3	0.171	0.000
4	-	0.000
5	-	-
6	0.211	-
7	0.244	0.000
8	0.292	0.000
9	0.292	0.000

2.16. 2M $(NH_4)_2SO_4$, 4.0M NH_4OH pH 10. 25cm^3 of 10ppm Cr(VI). (Long Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.01	12	0.397
2	0.022	13	0.312
3	0.033	14	0.256
4	0.124	15	0.209
5	0.355	16	0.135
6	0.560	17	0.149
7	0.696	18	0.098
8	0.717	19	0.086
9	0.705	20	0.064
10	0.304	21	0.054
11	0.228	22	0.057

2.17. 2M $(NH_4)_2SO_4$, 2M NH_4OH pH 10. 25cm^3 of 10ppm Cr(VI). (Long Column).

Fraction	Abs.	Fraction	Abs.	Fraction	Abs.	Farction	Abs.
1	0.039	12	0.919	23	0.930	34	0.423
2	0.039	13	0.937	24	0.856	35	0.392
3	0.039	14	1.043	25	0.836	36	0.378
4	0.039	15	1.107	26	0.771	37	0.378
5	0.050	16	1.142	27	0.659	38	0.283
6	0.121	17	1.122	28	0.632	39	0.248
7	0.305	18	1.108	29	0.608	40	0.208

8	0.483	19	1.067	30	0.571	41	0.149
9	0.662	20	1.011	31	0.510	42	0.116
10	0.766	21	0.991	32	0.480	43	0.087
11	0.816	22	0.958	33	0.471	44	0.078

2.18. 2M $(NH_4)_2SO_4$, 0.8M NH_4OH pH 10. 25cm^3 of 10ppm Cr(VI). (Long Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.000	16	0.915
2	0.000	17	0.878
3	0.000	18	0.862
4	0.001	19	0.832
5	0.044	20	0.794
6	0.440	21	0.737
7	0.728	22	0.682
8	0.842	23	0.629
9	0.922	24	0.581
10	0.987	25	0.553
11	0.990	26	0.530
12	1.002	27	0.510
13	1.002	28	0.474
14	0.979	29	0.428
15	0.957	30	0.404

2.19. 2M $(NH_4)_2SO_4$, 8.0M NH_4OH pH 10. 25cm^3 of 10ppm Cr(VI). (Long Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.000	13	0.244
2	0.000	14	0.122
3	0.000	15	0.118
4	0.247	16	0.120
5	2.079	17	0.068
6	2.177	18	0.108
7	2.144	19	0.083
8	1.972	20	0.035
9	0.707	21	0.031
10	0.510	22	0.032
11	0.045	23	0.016
12	0.097	24	0.000
		25	0.000

2.20. 2M $(NH_4)_2SO_4$, 4.0M NH_4OH pH 11.9. 25cm^3 of 10ppm Cr(VI). (long Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.032	13	0.375
2	0.033	14	0.399
3	0.036	15	0.407
4	0.037	16	0.420
5	0.038	17	0.453
6	0.102	18	0.458
7	0.212	19	0.438

8	0.277	20	0.429
9	0.360	21	0.410
10	0.393	22	0.358
11	0.170	23	0.381
12	0.405	24	0.299

2.21. 2M $(NH_4)_2SO_4$, 4.0M NH_4OH pH 9.0. 25cm^3 of 10ppm Cr(VI). (Long Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.001	11	0.675
2	2.250	12	0.605
3	2.263	13	0.536
4	2.254	14	0.446
5	2.170	15	0.377
6	1.863	16	0.331
7	1.546	17	0.289
8	1.345	18	0.265
9	1.115	19	0.227
10	0.858	20	0.206

2.22. 2M $(NH_4)_2SO_4$, 4.0M NH_4OH pH 10. 25cm^3 of 10ppm Cr(VI).(Short Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.009	10	1.157
2	0.351	11	0.963
3	6.84	12	0.246
4	9.28	13	0.785
5	7.06	14	0.677
6	5.06	15	0.552
7	1.3	16	0.503
8	0.208	17	0.451
9	0.101	18	0.384

2.23. 2M $(NH_4)_2SO_4$, 4.0M NH_4OH pH 9.0. 25cm^3 of 1ppm Cr(VI). (Short Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.008	11	0.077
2	0.011	12	0.064
3	0.123	13	0.059
4	0.569	14	0.041
5	0.398	15	0.036
6	0.296	16	0.032
7	0.176	17	0.030
8	0.132	18	0.026
9	0.103	19	0.026
10	0.083	20	0.023

2.24. 2M $(NH_4)_2SO_4$, 4.0M NH_4OH pH 9.0. 25cm^3 of 100ppb Cr(VI). (Short Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.005	11	0.011
2	0.009	12	0.010
3	0.035	13	0.009
4	0.031	14	0.009

5	0.025	15	0.009
6	0.022	16	0.010
7	0.015	17	0.010
8	0.014	18	0.009
9	0.013	19	0.009
10	0.011	20	0.010

2.25. 2M $(NH_4)_2SO_4$,4.0M NH_4OH pH 10. 1 dm^3 of 5ppb Cr(VI). (Short Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.003	11	0.013
2	0.004	12	0.011
3	0.016	13	0.013
4	0.025	14	0.009
5	0.021	15	0.010
6	0.015	16	0.013
7	0.014	17	0.011
8	0.010	18	0.011
9	0.009	19	0.013
10	0.015	20	0.012

2.26. 2M $(NH_4)_2SO_4$,4.0M NH_4OH pH 10. Chromium Enriched Yeast Extract. (Short Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.000	11	0.006
2	0.002	12	0.005
3	0.005	13	0.004
4	0.009	14	0.004
5	0.012	15	0.002
6	0.014	16	0.002
7	0.012	17	0.001
8	0.011	18	0.003
9	0.010	19	0.004
10	0.008	20	0.004

2.27. 2M $(NH_4)_2SO_4$,4.0M NH_4OH pH 10. Non-Enriched Yeast Extract. (Short Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.020	11	0.020
2	0.025	12	0.019
3	0.020	13	0.020
4	0.019	14	0.020
5	0.019	15	0.020
6	0.020	16	0.020
7	0.019	17	0.020
8	0.020	18	0.015
9	0.020	19	0.015
10	0.020	20	0.016

2.28. 2M $(NH_4)_2SO_4$,4.0M NH_4OH pH 10. Non-Enriched Yeast + 20 ppm Cr(VI). (Short Column).

Fraction	Absorbance	Fraction	Absorbance
1	0.018	11	0.042
2	0.031	12	0.040
3	0.029	13	0.035
4	0.028	14	0.039
5	0.026	15	0.037
6	0.033	16	0.031
7	0.032	17	0.043
8	0.033	18	0.047
9	0.030	19	0.032
10	0.043	20	0.021

2.29. 0.1M H_2SO_4,0.1M Ascorbic Acid.25cm^3 of 1ppm Cr(VI). Eluted Immediately.

Fraction	Absorbance	Fraction	Absorbance
1	0.001	8	0.352
2	0.007	9	0.269
3	0.013	10	0.241
4	0.653	11	0.202
5	0.625	12	0.180
6	0.615	13	0.163
7	0.431		

2.30. 0.1M H_2SO_4,0.1M Ascorbic Acid. 25cm^3 of 1ppm Cr(VI). Eluted After 1 Hour.

Fraction	Absorbance	Fraction	Absorbance
1	0.024	11	0.324
2	0.025	12	0.167
3	0.020	13	0.197
4	0.026	14	0.163
5	0.038	15	0.151
6	0.134	16	0.104
7	0.264	17	0.134
8	0.293	18	0.106
9	0.299	19	0.105
10	0.367	20	0.083

2.31. 0.1M H_2SO_4,0.1M Ascorbic Acid.25cm^3 of 100ppb Cr(VI).Eluted After 1 Hour.

Fraction	Absorbance	Fraction	Absorbance
1	0.031	11	0.273
2	0.041	12	0.224
3	0.028	13	0.213
4	0.034	14	0.156
5	0.036	15	0.149
6	0.110	16	0.116
7	0.157	17	0.102
8	0.209	18	0.104
9	0.258	19	0.107
10	0.251	20	0.064

2.32. 0.1M H_2SO_4,0.1M Ascorbic Acid.100cm^3 of 10ppb Cr(VI).Eluted After 1 Hour.

Fraction	Absorbance	Fraction	Absorbance
1	0.020	11	0.257
2	0.013	12	0.227
3	0.015	13	0.141
4	0.015	14	0.126
5	0.007	15	0.105
6	0.009	16	0.090
7	0.032	17	0.083
8	0.087	18	0.071
9	0.132	19	0.059
10	0.173	20	0.063

2.33. 0.1M H_2SO_4,0.1M Ascorbic Acid. 1 dm^3 of 1ppb Cr(VI). Eluted After 1 Hour.

Fraction	Absorbance	Fraction	Absorbance
1	0.031	11	0.260
2	0.048	12	0.284
3	0.044	13	0.293
4	0.039	14	0.323
5	0.030	15	0.306
6	0.050	16	0.275
7	0.065	17	0.269
8	0.151	18	0.261
9	0.158	19	0.252
10	0.147	20	0.212

2.34. 0.1M H_2SO_4,0.1M Ascorbic Acid. Chromium Enriched Yeast Extract. Eluted After 1 Hour.

Fraction	Absorbance	Fraction	Absorbance
1	0.131	11	0.563
2	0.061	12	0.435
3	0.051	13	0.420
4	0.085	14	0.475
5	0.554	15	0.617
6	0.749	16	0.711
7	0.539	17	0.766
8	0.867	18	0.468
9	0.836	19	0.473
10	0.596	20	0.410

2.35. 0.1M H_2SO_4,0.1M Ascorbic Acid. Non-Enriched Yeast Extract. Eluted After 1 Hour.

Fraction	Absorbance	Fraction	Absorbance
1	0.021	11	0.208
2	0.012	12	0.138
3	0.009	13	0.070
4	0.015	14	0.104
5	0.042	15	0.088

6	0.035	16	0.106
7	0.139	17	0.075
8	0.084	18	0.079
9	0.074	19	0.047
10	0.184	20	0.060

2.36. Elution of Cr(III) From Chromium Mixture.

Fraction	Absorbance	Plotted
1	-0.0051	0.0024
2	-0.0053	0.0022
3	-0.0061	0.0014
4	-0.0063	0.0012
5	-0.0050	0.0025
6	0.0149	0.0224
7	0.0477	0.0552
8	0.0062	0.0137
9	-0.0008	0.0067
Eluent	-0.0075	

2.37. Elution of Cr(VI) From Chromium Mixture.

Fraction	Absorbance	Fraction	Absorbance
1	0.007	11	0.005
2	0.009	12	0.002
3	0.029	13	0.001
4	0.044	14	0.002
5	0.031	15	0.002
6	0.020	16	0003
7	0.013	17	0.002
8	0.003	18	0.002
9	0.005	19	0.004
10	0.005	20	0.002

2.38. Elution Of Cr(III) From Cr(III) Standard.

Fraction	Absorbance 1	Absorbance 2	Average Abs.	Plotted
1	-0.0025	-0.0020	-0.00225	0.0040
2	-0.0025	-0.0031	-0.0028	0.00345
3	0.0019	0.0012	0.00155	0.0078
4	0.0373	0.0325	0.0349	0.04115
5	0.0646	0.0644	0.0645	0.07075
6	0.0132	0.0132	0.0132	0.01945
7	0.0067	0.0063	0.0065	0.01275
8	0.0023	0.0027	0.0025	0.00875
9	0.0009	0.0006	0.00075	0.007
10	-0.0015	-0.0014	-0.00145	0.0048
Eluent	-0.0065	-0.0060	-0.00625	

2.39. Elution Of Cr(VI) From Cr(III) Standard.

Fraction	Absorbance	Fraction	Absorbance
1	0.019	8	0.004
2	0.009	9	0.003
3	0.005	10	0.003
4	0.005	11	0.002
5	0.003	12	0.002
6	0.002	13	0.003
7	0.003	14	0.003
		15	0.004

2.40. Elution Of Cr(III) From Chromium Enriched Yeast Extract.

Fraction	Absorbance 1	Absorbance 2	Average Abs.	Plotted
1	0.0000	-0.0025	-0.00125	0.00440
2	-0.0025	-0.0036	-0.00305	0.00260
3	-0.0038	-0.0039	-0.00385	0.00180
4	0.0033	0.0030	0.00315	0.00880
5	0.0516	0.0498	0.05070	0.05635
6	0.0245	0.0260	0.02530	0.03095
7	0.0104	0.0097	0.01000	0.01575
8	0.0044	0.0300	0.00370	0.00935
9	0.0017	0.0021	0.00190	0.00755
10	-0.0022	-0.0029	-0.00255	0.00310
Eluent	-0.0058	-0.0055	-0.00565	

2.41.Elution Of Cr(VI) From Chromium Enriched Yeast Extract.

Fraction	Absorbance	Fraction	Absorbance
1	-0.002	8	-0.006
2	0.009	9	-0.002
3	-0.004	10	-0.001
4	-0.005	11	-0.001
5	-0.006	12	-0.002
6	-0.007	13	-0.003
7	-0.006	14	-0.002
		15	-0.001

2.42. Reduction Of Cr(VI) By 0.01M Ascorbic Acid.

Time / seconds	Absorbance	Time / seconds	Absorbance
20	1.134	130	0.770
30	1.088	140	0.746
40	1.064	150	0.718
50	1.026	160	0.696
60	1.003	170	0.669
70	0.961	180	0.648
80	0.927	190	0.623
90	0.889	200	0.600
100	0.863	210	0.577
110	0.833	220	0.562
120	0.803	230	0.536

2.43. Reduction Of Cr(VI) By 0.02M Ascorbic Acid.

Time / seconds	Absorbance	Time / seconds	Absorbance
20	1.044	130	0.529
30	0.988	140	0.496
40	0.933	150	0.467
50	0.879	160	0.438
60	0.829	170	0.408
70	0.755	180	0.382
80	0.729	190	0.359
90	0.683	200	0.339
100	0.643	210	0.317
110	0.603	220	0.298
120	0.563	230	0.278
		240	0.263

2.44. Reduction Of Cr(VI) By 0.03M Ascorbic Acid.

Time / seconds	Absorbance	Time / seconds	Absorbance
20	1.080	130	0.333
30	0.988	140	0.299
40	0.8984	150	0.272
50	0.799	160	0.244
60	0.717	170	0.221
70	0.641	180	0.200
80	0.574	190	0.182
90	0.516	200	0.168
100	0.460	210	0.152
110	0.416	220	0.139
120	0.369	230	0.130
		240	0.119

2.45. Reduction Of Cr(VI) By 0.04M Ascorbic Acid.

Time / seconds	Absorbance	Time / seconds	Absorbance
20	1.034	130	0.222
30	0.906	140	0.195
40	0.789	150	0.172
50	0.681	160	0.152
60	0.589	170	0.135
70	0.506	180	0.122
80	0.439	190	0.113
90	0.380	200	0.101
100	0.333	210	0.091
110	0.286	220	0.085
120	0.251	230	0.080
		240	0.075

2.46. Reduction Of Cr(VI) By 0.05M Ascorbic Acid.

Time / seconds	Absorbance	Time / seconds	Absorbance
20	0.933	130	0.139
30	0.775	140	0.123
40	0.634	150	0.112
50	0.520	160	0.100
60	0.432	170	0.090
70	0.358	180	0.083
80	0.301	190	0.077
90	0.254	200	0.072
100	0.215	210	0.068
110	0.185	220	0.065
120	0.162	230	0.063
		240	0.060

2.47. Reduction Of Cr(VI) By 0.06M Ascorbic Acid.

Time / seconds	Absorbance	Time / seconds	Absorbance
20	0.901	130	0.094
30	0.700	140	0.085
40	0.545	150	0.079
50	0.428	160	0.075
60	0.338	170	0.072
70	0.268	180	0.069
80	0.214	190	0.067
90	0.173	200	0.066
100	0.143	210	0.063
110	0.120	220	0.065
120	0.104	230	0.063
		240	0.062

2.48. Determination Of The Second Order Rate Constant

K_{obs} / s^{-1}	[Ascorbate]	k_{obs} / s^{-1}	[Ascorbate]
0.00356	0.01	0.01377	0.04
0.00629	0.02	0.01718	0.05
0.0106	0.03	0.02199	0.06

2.49. Reduction Of 200ppm Cr(VI) By Enriched Yeast In Acidic Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0	1.021	100	6	0.315	30.85211
1	0.713	69.8335	7	0.284	27.81587
2	0.605	59.25563	8.5	0.277	27.13026
3	0.548	53.67287	10	0.256	25.07346
4	0.439	42.99706	23	0.071	6.95397
5	0.363	35.55338			

2.50. Reduction Of 200ppm Cr(VI) By Enriched Yeast In Acidic Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
1	0.940	100.00000	7	0.336	35.74468
2	0.868	92.34043	8	0.313	33.29787
3	0.821	87.34043	9	0.288	30.63830
4	0.565	60.10638	10	0.249	26.48936
5	0.532	56.59574	11	0.197	20.95745
6	0.490	52.12766			

2.51. Reduction Of 100ppm Cr(VI) By Enriched Yeast In Acidic Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	2.003	100.00000	4.0	0.877	43.78432
0.5	1.378	68.79680	5.0	0.831	41.48777
1.0	1.225	61.15826	6.0	0.724	36.14578
1.5	1.183	59.06141	7.0	0.600	29.95507
2.0	1.147	57.26410	8.0	0.530	26.46031
2.5	1.089	54.36845	9.0	0.489	24.41338
3.0	1.042	52.02197	11.0	0.420	20.96855
			24.0	0.240	11.98203

2.52. Reduction Of 100ppm Cr(VI) By Enriched Yeast In Acidic Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.00	2.005	100.00000	2.50	0.994	49.57606
0.25	1.725	86.03491	4.00	0.786	39.20200
0.50	1.355	67.58105	5.00	0.689	34.36409
1.00	1.302	64.93766	6.00	0.622	31.02244
1.50	1.105	55.11222	7.50	0.600	29.92519
2.00	1.017	50.72319	9.00	0.528	26.33416

2.53. Reduction Of 50ppm Cr(VI) By Enriched Yeast In Acidic Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.00	0.995	100.00000	3.00	0.360	36.1809
0.25	0.729	73.26633	4.00	0.319	32.0603
0.50	0.606	60.90452	5.50	0.276	27.73869
0.75	0.530	53.26633	6.50	0.239	24.02010
1.00	0.483	48.54271	7.50	0.196	19.69849
1.25	0.428	43.01508	8.50	0.131	13.16583
1.50	0.416	41.80905	11.0	0.118	11.8593
2.00	0.377	37.88945			

2.54. Reduction Of 50ppm Cr(VI) By Enriched Yeast In Acidic Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.00	1.057	100.00000	3.00	0.373	35.28855
0.25	0.664	62.81930	4.00	0.325	30.74740
0.50	0.601	56.85904	5.00	0.256	24.21949
0.75	0.578	54.68307	6.00	0.217	20.52980
1.00	0.574	54.30464	7.00	0.209	19.77294
1.50	0.528	49.95270	8.00	0.199	18.82687

2.00	0.450	42.57332	9.00	0.174	16.46168
2.50	0.391	36.99149	24.0	0.125	11.82592

2.55. Reduction Of 200ppm Cr(VI) By Enriched Yeast In Neutral Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.00	1.678	100.00000	5.00	1.496	89.15375
0.25	1.390	82.83671	6.00	1.491	88.85578
0.50	1.717	102.3242	7.50	1.791	106.73421
0.75	1.619	96.48391	9.50	1.762	105.00596
1.00	1.775	105.78069	10.00	1.516	90.34565
1.50	1.765	105.18474	23.75	1.886	112.39571
2.00	1.790	106.67461	27.50	2.370	141.23957
3.00	1.760	104.88677	32.50	2.516	149.94041
4.00	1.544	92.01430			

2.56. Reduction Of 200ppm Cr(VI) By Enriched Yeast In Neutral Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.00	1.484	100.00000	6.00	1.585	106.80593
0.25	1.393	93.86792	7.50	1.628	109.70350
0.75	1.376	92.72237	8.50	1.669	112.46631
1.00	1.136	76.54987	10.00	1.668	112.39892
1.50	1.449	97.64151	23.75	1.561	105.18868
2.00	1.448	97.57412	27.50	1.556	104.85175
3.00	1.494	100.67385	32.50	1.533	103.30189
4.00	1.606	108.22102	48.00	1.627	109.63612
5.00	1.385	93.32884			

2.57. Reduction Of 100ppm Cr(VI) By Enriched Yeast In Neutral Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	0.900	100.00000	7.5	0.669	74.33333
1.0	0.731	81.22222	9.5	0.731	81.22222
2.0	0.699	77.66667	24.0	0.737	81.88889
3.0	0.641	71.22222	29.0	0.707	78.55556
4.5	0.643	71.44444	48.0	0.607	67.44444
6.0	0.607	67.44444	120.0	1.083	120.33333

2.58. Reduction Of 100ppm Cr(VI) By Enriched Yeast In Neutral Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	0.965	100.0000	7.0	1.006	104.2487
1.0	0.840	87.0466	8.5	0.980	101.5544
2.5	0.956	99.0673	24.0	0.989	102.4870
3.5	0.941	97.5129	48.0	0.532	15.26012
5.0	0.930	96.3731	120.0	0.715	74.0933

2.59. Reduction Of 25ppm Cr(VI) By Enriched Yeast In Neutral Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	2.003	100.00000	7.5	1.841	91.91213
1.0	1.731	86.42037	8.5	2.072	103.44483
2.0	1.980	98.85172	9.5	2.084	104.04393
3.0	1.950	97.35397	72.0	1.621	80.92861
4.5	2.030	101.34798	96.0	1.769	88.31752
5.5	1.838	91.76236	120.0	1.772	88.46730
6.5	1.957	97.70344			

2.60. Reduction Of 25ppm Cr(VI) By Enriched Yeast In Neutral Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	1.704	100.00000	7.5	1.748	102.58216
1.0	1.526	89.55399	8.5	1.931	113.3216
2.0	1.450	85.0939	9.5	1.672	98.12207
3.0	1.672	98.12207	72.0	1.381	81.04460
4.5	1.756	103.05164	96.0	1.943	114.02582
5.5	1.866	109.50704	120.0	1.874	109.97653
6.5	1.718	100.8216			

2.61. Reduction Of 200ppm Cr(VI) By Enriched Yeast In Basic Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	1.613	100.00000	6.5	1.287	79.78921
1.0	1.600	99.19405	7.5	1.152	71.41971
2.0	1.256	77.86733	8.5	1.345	83.38500
3.0	1.113	69.00186	24.0	1.361	84.37694
4.5	1.210	75.01550	48.0	1.232	76.37942
5.5	1.444	89.52263	72.0	1.625	100.74396

2.62. Reduction Of 200ppm Cr(VI) By Enriched Yeast In Basic Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	1.598	100.00000	6.5	1.393	87.17146
1.0	1.447	90.55069	7.5	1.413	88.42303
2.0	1.544	96.62078	8.5	1.482	92.74093
3.0	1.328	83.10388	24.0	1.115	69.77472
4.5	1.225	76.65832	48.0	1.249	78.1602
5.5	1.875	17.33417	72.0	1.249	78.1602

2.63. Reduction Of 100ppm Cr(VI) By Enriched Yeast In Basic Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	0.986	100.00000	7.5	0.831	84.27992
1.0	0.550	55.78093	9.0	0.709	71.90669
2.0	0.423	42.90061	24.0	0.803	81.44016
3.0	0.666	67.54564	48.0	0.764	77.48479
4.5	0.647	65.61866	120.0	0.707	71.70385
5.5	0.624	63.28600	144.0	0.891	90.36511
6.5	0.677	68.66126	168.0	0.928	94.11765

2.64. Reduction Of 100ppm Cr(VI) By Enriched Yeast In Basic Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	0.889	100.00000	7.5	0.579	65.12936
1.0	0.582	65.46682	9.0	0.792	89.08886
2.0	0.545	61.30484	24.0	0.759	85.37683
3.0	0.798	89.76378	48.0	0.703	79.07762
4.5	0.649	73.00337	120.0	0.602	67.71654
5.5	0.576	64.79190	144.0	0.679	78.40270
6.5	0.533	59.95501	168.0	0.848	95.38808

2.65. Reduction Of 25ppm Cr(VI) By Enriched Yeast In Basic Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	1.168	100.00000	6.5	1.348	115.41096
1.0	1.151	98.54452	7.5	1.404	120.20548
2.0	1.059	90.66781	8.5	1.312	112.32877
3.0	1.062	90.92466	24.0	1.223	104.70890
4.5	1.136	97.26027	96.0	1.413	120.97603
5.5	0.106	94.69178	120.0	1.526	130.65068

2.66. Reduction Of 25ppm Cr(VI) By Enriched Yeast In Basic Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	1.394	100.00000	6.5	1.389	99.60689
1.0	1.383	99.21090	7.5	1.481	106.24103
2.0	1.299	93.18508	8.5	1.541	110.54519
3.0	1.232	88.37877	24.0	1.368	98.13486
4.5	1.128	80.91822	96.0	1.147	82.28121
5.5	1.277	91.60689	120.0	1.611	115.56671

2.67. Reduction Of 100ppm Cr(VI) By Non-Enriched Yeast In Acidic Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.00	1.989	100.00000	5.00	0.984	44.94721
0.25	1.481	74.45953	6.00	0.827	41.57868
0.50	1.423	71.54349	7.00	0.716	35.99799
0.75	1.386	69.68326	8.00	0.662	33.28306
1.25	1.364	68.57717	9.00	0.649	32.62946
1.75	1.306	65.66114	11.00	0.522	26.24434
3.50	1.064	53.49422	24.00	0.131	6.58622

2.68. Reduction Of 100ppm Cr(VI) By Non-Enriched Yeast In Acidic Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.00	1.624	100.00000	6.00	0.798	49.13793
0.25	1.548	95.3202	7.00	0.749	46.12069
1.00	1.490	91.74877	8.00	0.619	38.11576
1.50	1.261	77.64778	9.00	0.600	36.94581
2.00	1.245	76.66256	11.00	0.452	27.83251
3.50	1.064	65.51724	24.00	0.116	7.14286
5.00	0.885	54.49507			

2.69. Reduction Of 100ppm Cr(VI) by Non-Enriched Yeast in Neutral Solution in air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	0.938	100.00000	6.0	0.881	93.92324
0.5	0.920	98.08102	7.5	0.903	96.26866
1.5	0.868	92.53731	9.0	0.898	95.73561
2.5	0.953	101.59915	24.0	0.956	101.91898
3.5	0.900	95.94883	48.0	0.815	86.88699
5.0	0.817	87.10021	120.0	0.702	74.84009

2.70. Reduction Of 100ppm Cr(VI) by Non-Enriched Yeast in Neutral Solution in N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	0.624	100.00000	6.0	0.890	142.62821
0.5	0.172	27.56410	7.5	0.925	148.23718
1.5	0.924	148.07692	9.0	0.881	141.18590
2.5	0.796	127.56410	24.0	0.891	142.78846
3.5	0.738	118.26923	48.0	0.892	142.94872
5.0	0.849	136.05769	120.0	0.837	134.13462

2.71. Reduction Of 100ppm Cr(VI) By Non-Enriched Yeast In Basic Solution In Air.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	0.935	100.00000	6.0	0.903	96.57754
1.0	0.918	98.18182	7.5	0.922	98.60963
2.0	0.923	98.71658	9.0	0.984	105.24064
3.5	0.934	99.89305	24.0	0.799	85.45455
5.0	0.978	104.59893	96.0	0.813	86.95187

2.72. Reduction Of 100ppm Cr(VI) By Non-Enriched Yeast In Basic Solution In N_2.

Time/hours	Absorbance	%A	Time/hours	Absorbance	%A
0.0	1.054	100.00000	6.0	0.974	92.40987
1.0	0.804	76.28083	7.5	0.939	89.08918
2.0	0.927	87.95066	9.0	0.952	90.32258
3.5	0.931	88.33017	24.0	0.881	83.58634
5.0	1.055	100.09488	96.0	0.871	82.63757

2.73.Redox Potentials of 1.0M H_2SO_4.

Time/second	Potential / V	Time/second	Potential / V	Time/second	Potential / V
1	0.806	20	0.708	38	0.703
2	0.807	21	0.706	39	0.703
3	0.806	22	0.707	40	0.704
4	0.8	23	0.706	41	0.706
5	0.791	24	0.707	42	0.706
6	0.779	25	0.709	43	0.705
7	0.778	26	0.709	44	0.705
8	0.771	27	0.707	45	0.705
9	0.766	28	0.706	46	0.706
10	0.745	29	0.707	47	0.707
11	0.729	30	0.706	48	0.707
12	0.72	31	0.707	49	0.709
13	0.711	32	0.707	50	0.709

14	0.702	33	0.707	51	0.709
15	0.703	34	0.706	52	0.707
16	0.706	35	0.706	53	0.708
17	0.706	36	0.704	54	0.708
18	0.706	37	0.706	55	0.707
19	0.706	38	0.704		

2.74. Redox Potentials of 0.1M H_2SO_4.

Time/second	Potential / V	Time/second	Potential / V	Time/second	Potential / V
0	725.6	40	661.5	80	659.7
5	704.1	45	660.6	85	659.7
10	694.9	50	660.3	90	659.8
15	685.4	55	659.3	95	660.1
20	677.3	60	659.7	100	660
25	671	65	659.5	105	660.1
30	666.4	70	659.7	110	660.4
35	662.1	75	659.4	115	660.5
				120	660.7

2.75. Redox Potentials of 0.01M H_2SO_4.

Time/second	Potential / V	Time/second	Potential / V	Time/second	Potential / V
1	0.662	20	0.608	38	0.588
2	0.661	21	0.606	39	0.589
3	0.66	22	0.605	40	0.585
4	0.658	23	0.603	41	0.586
5	0.659	24	0.601	42	0.586
6	0.655	25	0.597	43	0.585
7	0.652	26	0.597	44	0.585
8	0.649	27	0.597	45	0.583
9	0.646	28	0.597	46	0.586
10	0.639	29	0.595	47	0.584
11	0.629	30	0.595	48	0.582
12	0.627	31	0.595	49	0.581
13	0.622	32	0.594	50	0.58
14	0.622	33	0.592	51	0.581
15	0.621	34	0.591	52	0.582
16	0.616	35	0.591	53	0.581
17	0.615	36	0.59	54	0.582
18	0.613	37	0.588	55	0.582
19	0.61	38	0.588	56	0.581

2.76.Redox Potentials of 0.1M Tris-NaOH pH 13.

Time/second	Potential / V	Time/second	Potential / V	Time/second	Potential / V
0	-0.133	40	-0.158	80	-0.155
5	-0.132	45	-0.163	85	-0.155
10	-0.15	50	-0.157	90	-0.156
15	-0.193	55	-0.154	95	-0.155
20	-0.162	60	-0.155	100	-0.156
25	-0.171	65	-0.155	105	-0.157

30	-0.16	70	-0.154	110	-0.156
35	-0.156	75	-0.153	115	-0.156
				120	-0.156

2.77. Redox Potentials of 1000ppm Cr(VI) Plus 1g of Chromium Enriched Yeast.

$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V
0	437.7	10	753.1	20	780.7
0.5	569.5	10.5	755	20.5	781.5
1	608.5	11	757.2	21	782.3
1.5	634.5	11.5	759.4	21.5	783.3
2	655.8	12	761.3	22	785.4
2.5	672.2	12.5	763.3	22.5	785.9
3	684.6	13	764.9	23	785.9
3.5	696.6	13.5	766.3	23.5	786.7
4	702.8	14	768.2	24	787.3
4.5	709.6	14.5	769.6	24.5	787.7
5	716	15	770.7	25	788.1
5.5	720.3	15.5	772.1	25.5	789
6	725.5	16	773.5	26	790.2
6.5	729.6	16.5	774.3	26.5	791.1
7	733.3	17	775.6	27	791.9
7.5	736.3	17.5	776.7	27.5	792.1
8	739.5	18	777.7	28	792.5
8.5	742.4	18.5	779.1	28.5	793.1
9	745.1	19	779.9	29	793.6
9.5	748.4	19.5	779.9	29.5	794.5

2.78. Redox Potentials of 100ppm Cr(VI) Plus 1g of Chromium Enriched Yeast.

$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V
0	450.2	10	617	20	654.8
0.5	474.2	10.5	620.7	20.5	656.7
1	498.1	11	623.1	21	657.8
1.5	518.2	11.5	625.7	21.5	659.4
2	531.8	12	628	22	661.5
2.5	541.8	12.5	630.2	22.5	662.5
3	552.3	13	632.5	23	663.7
3.5	560.2	13.5	633.9	23.5	664.8
4	566.3	14	636.2	24	665.6
4.5	573.2	14.5	638.4	24.5	667.3
5	579.8	15	640.9	25	668.3
5.5	584.1	15.5	642.5	25.5	669.6
6	589.6	16	644.6	26	671.2
6.5	593.4	16.5	645.7	2.5	672.3
7	598.3	17	647.6	27	673.5
7.5	601.4	17.5	649.1	27.5	674.5
8	605	18	650.3	28	675.4
8.5	608.8	18.5	652	28.5	676
9	611.3	19	653.9	29	676.9
9.5	614.3	19.5	654.4	29.5	678

2.79. Redox Potentials of 10ppm Cr(VI) Plus 1g of Chromium Enriched Yeast.

$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V
0	438	11.5	510.2	23	537.9
0.5	439.9	12	512.1	23.5	538.9
1	444.5	12.5	513.9	24	539.8
1.5	448.8	13	515.5	24.5	540.9
2	452.9	13.5	517.1	25	541.5
2.5	457	14	518.5	25.5	542.3
3	464.1	14.5	519.8	26	543.3
3.5	468	15	521	2.5	544.2
4	472.1	15.5	522.6	27	545.1
4.5	475.6	16	524.4	27.5	546.4
5	479.5	16.5	525.4	28	547.3
5.5	481.9	17	527	28.5	548.6
6	483.7	17.5	528.1	29	549
6.5	487.5	18	528.7	29.5	550
7	489.5	18.5	529.5	30	551.2
7.5	491.4	19	530.1	30.5	552
8	494.1	19.5	530.9	31	552.7
8.5	496.2	20	531.7	31.5	553.7
9	499.9	20.5	532.6	32	554.7
9.5	502.8	21	533.5	32.5	555.6
10	504.4	21.5	534.6	33	556.7
10.5	506.8	22	535.4	33.5	557.4
11	508.2	22.5	536.6	34	558.6

2.80. Redox Potentials of 1ppm Cr(VI) Plus 1g of Chromium Enriched Yeast.

$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V
0	464.1	14	506.2	28	531.3
0.5	467.5	14.5	507.5	28.5	531.8
1	469.8	15	508.4	29	532.4
1.5	472.9	15.5	509.1	29.5	533
2	474.1	16	510.5	30	533.4
2.5	476.5	16.5	511.3	30.5	533.9
3	478.3	17	512.9	31	534.2
3.5	480.3	17.5	514.3	31.5	534.6
4	482	18	515.5	32	534.9
4.5	483.2	18.5	516.7	32.5	535.2
5	484.4	19	517.8	33	535.5
5.5	485.6	19.5	519	33.5	535.8
6	487.2	20	519.8	34	536.2
6.5	488.5	20.5	521.2	34.5	536.4
7	490.2	21	521.9	35	536.6
7.5	194.4	21.5	522.6	35.5	536.9
8	493	22	523.5	36	537.1
8.5	493.9	22.5	524.4	36.5	537.4
9	495.5	23	525.4	37	537.7
9.5	496.9	23.5	526	37.5	537.9

10	498.4	24	526.5	38	538.1
10.5	499.3	24.5	527.3	38.5	538.3
11	500.4	25	527.8	39	538.5
11.5	501.2	25.5	528.4	39.5	538.6
12	502.1	26	529.1	40	538.7
12.5	502.9	2.5	529.6	40.5	538.9
13	503.8	27	530.3	41	539.1
13.5	504.9	27.5	530.9	41.5	539.2

2.81.Redox Potentials of 0.1ppm Cr(VI) Plus 1g of Chromium Enriched Yeast.

$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V
0	459.4	12	489.4	24	495.5
0.5	459.7	12.5	490.1	24.5	495.6
1	461.5	13	490.7	25	495.8
1.5	462.5	13.5	491.2	25.5	496
2	463.5	14	491.8	26	496.1
2.5	465.6	14.5	492.2	2.5	496.3
3	467.4	15	492.7	27	496.4
3.5	468.8	15.5	493	27.5	496.6
4	469.6	16	493	28	496.8
4.5	471.1	16.5	493.2	28.5	496.9
5	472.4	17	493.2	29	497.1
5.5	474	17.5	493.4	29.5	497.2
6	475.4	18	493.6	30	497.3
6.5	477.6	18.5	493.8	30.5	497.4
7	478.9	19	493.9	31	497.6
7.5	480.1	19.5	494.2	31.5	497.7
8	481.4	20	494.4	32	497.7
8.5	482.8	20.5	494.6	32.5	497.8
9	483.8	21	494.7	33	497.9
9.5	484.6	21.5	494.8	33.5	497.9
10	485.7	22	494.9	34	497.9
10.5	486.6	22.5	495.1	34.5	498
11	487.8	23	495.2	35	498
11.5	488.7	23.5	495.3	35.5	498.1

2.82.Redox Potentials of 0.01ppm Cr(VI) Plus 1g of Chromium Enriched Yeast.

$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V
0	443.4	12	469	24	477
0.5	443.6	12.5	469.7	24.5	477.1
1	444.8	13	470.3	25	477.2
1.5	446.1	13.5	470.7	25.5	477.3
2	447.6	14	471.3	26	477.4
2.5	449.2	14.5	471.9	2.5	477.4
3	450.4	15	472.6	27	477.5
3.5	452	15.5	473.1	27.5	477.5
4	453.5	16	473.5	28	477.6
4.5	455.5	16.5	473.9	28.5	477.6

5	456.4	17	474.3	29	477.7
5.5	457.5	17.5	474.8	29.5	4777.7
6	458.7	18	475.1	30	477.7
6.5	460.3	18.5	475.4	30.5	477.8
7	461.3	19	475.7	31	477.8
7.5	462.1	19.5	475.9	31.5	477.8
8	463.2	20	476.2	32	477.9
8.5	464.1	20.5	476.3	32.5	477.9
9	464.8	21	476.4	33	477.9
9.5	465.6	21.5	476.5	33.5	477.9
10	466.2	22	476.6	34	478
10.5	466.9	22.5	476.7	34.5	478
11	467.5	23	476.8	35	478
11.5	468.2	23.5	476.9		

2.83.Redox Potenials of 0.01ppm Cr(VI) without the Addition of Yeast.

$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V	$Cr(VI)/cm^3$	Potential / V
0	569.4	14	582	28	601.2
1	569	15	583.2	29	603.7
2	569	16	584.4	30	604.9
3	569.3	17	585.7	31	606.2
4	570.1	18	586.7	32	606.2
5	571	19	588.3	33	607.3
6	572	20	590	34	608.4
7	573.2	21	591.3	35	609.6
8	574.3	22	592.5	36	610.7
9	575.4	23	594.2	37	611.9
10	576.6	24	595.4	38	613
11	577.9	25	597	39	614.1
12	579.3	26	598.4	40	615.3
13	580.5	27	599.8		

Bibliography.

Abraham, A. S., Brooks, B. A., and Eyleth, U. (1992), The Effect of Chromium Supplementation on Serum Glucose and Lipids in Patients with and without Non-Insulin Dependant Diabetes. *Metabolism*, **41**, 768-771.

Ahmad, S., Murthy, R. C., and Chandra, S. V. (March 1990). Chromium Speciation by Column Chromatography using a Direct Current Plasma Atomic Emission Spectrometer. *Analyst*, **115**, 287-289.

Allen, T. L. (March 1958). Microdetermination of Chromium with 1,5-DPC. *Analytical Chemistry*, **30**, No. 3, 447-450.

Alwens, W., and Jonas, W. (1938). Der Chromat Lunenkrebs. *Acta*, Union Int. Cancrum, **3**, 103.

Amdur, M. O., Daull, J., and Klaassen, C. D. (1991). Casarett and Doull's Toxicology. 4th Ed., *Pergamon Press, New York*, pp. 10, **36**, 157-158, 209-212.

Analytical Methods for Graphite Furnace Tube Atomisers. (January 1982). Published by Varian.

Arar, E. J., Long, S. E., Martin, T. D., and Gold, S. (1992). Determination of Hexavalent Chromium in Sludge Incinerator Emissions using Ion Chromatography

and Inductively Coupled Plasma Mass Spectroscopy. *Environ, Sci, Technol*, **26**, 1944-1950.

Babko, A. K., and Palii, L. A. (1950), *Zhur Anal. Khim*. **5**, 272.

Baetjer, A. M. (1950), Pulmonary Carcinoma in Chromate Workers, I. A. Review of the Literature and Report of Cases. *Arch. Ind. Hyg. Occup. Med*., **2**, 487-504.

Baetjer, A. M. (1950), Pulmonary Carcinoma in Chromate Workers, II. Incidence of Basis of Hospital Records. *Arch. Ind. Hyg. Occup. Med.*, **2**, 505-516.

Bartlett, R., and James, B. (1979), Behaviour of Chromium in Soils: III Oxidation. *J. Environ. Qual.*, **8**, No. 1.

Bartlett. R. J., and James, B. R. (1979), Oxidation of Chromium in Soils. *J. Environ. Qual*., **8**, 31-35.

Bose, M. (1954), The Reaction of Chromate with Diphenylcarbazide I. *Analytical Chimica Acta*, **10**, 201-208.

Bose, M. (19954), The Reaction of Chromate with Diphenylcarbazide II. *Analytical Chimica Acta*, **10**, 209-221.

Cieslak-Golonka, M. (1995), Toxic and Mutagenic Effects of Chromium (VI). A Review Polyhedron Report No. 61. *Polyhedron*. **15**, No. 21, 3667-3689.

Cohen, M.D., Kargaoin, B., Klein, C. B., and Costa, M. (1993), Mechanisms of Chromium Carcinogenicity and Toxicity. *Critical Reviews in Toxicology*, **23** (3): 255-281.

Connett, P. H., Wetterhahn, K.E. (1985), InVitro Reaction of the Carcinogenic Chromate with Cellular Thiols and Carboxylic Acids. *J. Am. Chem. Soc.*, **107**, 4282 - 4288.

Cutshall, N., Johnson, V., and Osterberg, C. (1966), Chromium-51 in Sea Water: *Chemistry Science*, **152**, 202-203.

De Andrade, J.C., Rocha, J. C., and Baccon, N. (February 1985), Sequential Spectrophotometric Determination of Chromium (III) and Chromium (VI) using Flow Injection Analysis. *Anaylst*, **110**, 197-199.

De Flora, S., and Wetterhahn, K., E. (1989), Mechanisms of Chromium Metabolism and Genotoxicity. *Life Chem. Rep.*, **7**, 169-244.

Demirata, B., Tor, I., Filik, H., and Afsar, H. (1996), Separation of Cr(III) and Cr(VI) using Melamine-Formaldehyde Resin and Determination of Both Species in Water by F.A.A.S.. *Fresenius J. Anal. Chem.* **356**:375-377.

Dixon, D. A., Sadler, N. P., Dasgupta, T. P., (1993), Oxidation of Biological Substrates by Chromium (VI). Part 1. Mechanism of the Oxidation of L – Ascorbic Acid in Aqueous Solution. *J. Chem. Soc. Dalton. Trans.*, 3489 – 3495.

Ehman, D. L., Anselmo, V. C., and Jenks, J. M. (1987), Determination of Low Levels of Airborne Chromium (VI) by Anion Exchange Treatment and Inductively Coulped Plasma Spectroscopy. *Spectroscopy*, **3**, 33-35.

Flores-Veles, L. M., Gutierrez-Ruiz, M. E., Reyes-Salas, O., Cram-Heydrich, S., and Baeza-Reyes, A. (1994), Separation of Cr(VI) in Soil Extracts by Polarographic Methods. *Intern. J. Environ. Anal. Chem.*, **61.** 177-187.

Gaspar, A., Posta, J., and Tothg, R. (November 1996), On-line Chromatographic Separation and Determination of Chromium (III) and Chromium (VI) with Preconcentration of the Chromium (III) using Potassium Hydrogen Phthalate, in Various Samples by Flame Atomic Adsorption Spectroscopy. *Journal of Analytical Atomic Spectroscopy*. **11**, 1067-1074.

Ghandour, M. A., El-Shantoury, S. A., Aly, A. M. M., and Ahmed, S., M. (1996), Adsorptive Cathodic Stripping Voltammetry Determination of Hexavalent Chromium. *Analytical Letters*, **29** (8), 1431-1445.

Glaser, E., and Halpern, G. (1929), Uber die Akivierung des Insulins-Durch Hefapressaft. *Biochem. Z.*, **207**, 377-383.

Goodgame, D. M. L., Joy. A. M. (1987), Formation of Chromium (V) during the Slow Reduction of Carcinogenic Chromium (VI) by Milk and Some of its Constituents. *Inorganica Chimica Acta*, **135**, L5 – L7.

Greenwood, N.N., and Earnshaw, A. (1984), *Chemistry of the Elements*. Peragamom Press Ltd..

Gupta, S.K. (1984), Importance of Soil Solution Composition in Deciding the Best Suitable Anaytical Criteria for Guidence on Maximum Tolerable Metal Load and in Assesing Bio-significance of Metals in Soil. *Schweiz, Landswirtsch, Forsch*, **23**, 209-225.

Guthrie, B. E., (1982), **Chapter 6**: The Nutritional Role of Chromium, in : Langard, S. (ed.): Biological and Environmental Aspects of Chromium, *Elservier Biomedical*, 1982, 117-148. Elsevier, Amsterdam.

Heringer Donmez, L. A.; Kallenberger, W. E. (1989), Soil Leachate: Determination of Hexavalent Chromium. *Jalca*, **84**.

Hoshi, S., Konuma, K., Sugawara, K., Uto, M., and Akatsuka, K. (1998), The Simple and Rapid Spectrophotometric Determination of Trace Chromium (VI) After Preconcentration as its Coloured Complex on Chitin. *Talanta*, **47**, 659-663.

Katz, S. A., and Salem, H. (1994), *The Biological and Environmental Chemistry of Chromium*. V. C. H..

Kieffer, F. (1979), Spurenelemente Steurin Die Gesundheit, *Sandoz Bull.*, **52**, 18-19.

Kilau, H. W., and Shah, I. D. (1984), Chromium-Bearing Waste Slag: Evaluation of Leachability when Exposed to Acid Precipitation, in Hazardous and Industrial Waste Management and Testing. ASTM STP 851, L. P. Jackson, A. R. Rohlik, and A. R. Conway, Eds., American Society for Testing and Materials, *Philadelphia*, 61-80.

Korolczuk, M., and Grabarczyk, M. (1998), Voltammetric Determination of Traces of Cr(VI) and Total Chromium using Catalytic Currents in the Presence of Nitrate. *Chem. Anal.* (Warsaw), **43**, 257.

Korolczuk, M., and Grabarczyk, M. (1998), Voltammetric Determination of Cr(VI) in a Flow System in the Presence of Diethylenetriamine-Pentaacetic Acid (DTPA) Following its Deposition in the Metallic State. *Analtytica Chimica Acta*, **387**, 97-102.

Korolczuk, M., and Grabarczyk, M. (1999), Voltammetric Determination of Traces of Cr(VI) in the Flow System in the Presence of Bipyridine. *Talanta*, **49**, 703-709.

Langard, S. (1982), **Chapter 4**: Chromium, in: Waldron, H.A. (ed.): *Metals in the Environmental*, pp. 111-132. Academic Press, London.

Li, Z., Shi, Y., Gao, P., Gu, X., and Zhou, T. (1997), Determination of Trace Chromium (VI) in Water by Graphite Furnace Atomic Absorption Spectroscopy after Preconcentration on a Soluble Membrane Filter. *Fresenius J Anal Chem*, **358**:519-522.

Lindsay, S. (1992). *High Performance Liquid Chromatography*. Published by John Wiley and Sons. New York.

Mertz, W., and Schwartz, K. (1955), Impaired Intravenous Glucose Tolerance as an Early Sign of Dietary Necrotic Liver Degeneration. *Arch, Biochem. Biophys*., **58**, 504-508.

Mertz, W. (1969), Chromium Occurrence and Function in Biological Systems. *Physiol. Rev.*, **49**, 163-239.

Naranjit, D., Thomassen, Y., and Van Loon, J. C. (1979), Development of a Procedure for Studies of the Chromium (III) and Chromium (VI) Contents of Welding Fumes. *Analytica Chimica Acta*, **110**, 307-312.

Newman, D. (1890), A Case of Adenocarcinoma of the Left Inferior Turbinate Body and Perforation of the Nasal Septum in the Person of a worker in Chrome Pigments. *Glasgow Med. J.*, **33**, 469-470.

O'Brien, P., and Woodbridge, N. (1997), A Study of the Kinetics of the Reduction of Chromate by Ascorbate under Aerobic and Anaerobic Conditions. *Polyhedron*. **16**, No. 12, 2081 – 2086.

Offenbacher, E. G., Spencer, H., Dowling, H. J., and Pi-Sunyer, F. X. (1986), Metabolic ChromiumBalances in Men. *Am. J. Clin. Nutr.*, **44**, 77-82.

Pacyna, J., and Nriagu, J. O. (1988), Atmospheric Emissions of Chromium from Natural and Anthropogenic Sources, in Chromium in the Natural and Human Environment. J. O. Nriagu and E. Nieboer, Eds., Wiley, New York, 116-117.

Padarauskas, A., Judzentiene, A., Naujalis, E., and Paliulionyte, V. (1998), On-Line Preconcentration and Deteermination of Chromium (VI) in Waters by High Performance Liquid Chromatography using Precolumn Complexation with 1,5-diphenylcarbazide. *Journal of Chromatography A*, **808**, 193-199.

Pankow, J. F., and Janaeur, G. E. (1974), Analysis for Chromium Traces in Natural Waters. Part I. Preconcentreation of Chromate from P.P.B. Levels in Aqueous Solutions by Ion Exchange. *Analytica Chimica Acta*, **69**, 97-104.

Perrin, D. D., and Dempsey, B. (1974), *Buffers for pH and Metal Ion Control. Chapman and Hall Laboratory Manuals*. Published by Chapman and Hall Ltd..

Pettine, M., Millero, F. J., and Noce, T. L. (1991), Chromium (III) Interactions in Seawater through its Oxidation Kinetics. *Mar. Chem.*, **34**, 29-46.

Pfeil, E. (1935), Lungentumoran als Berufskrankung in Chrima-tbetriehen. *Dtsch. Med. Wochenschr.*, **61**, 1197-1202.

Phifer, E. C. (1995), Determination of Chromium and Molybdenum in Medical Foods by Graphite Furnace Atomic Absorption Spectroscopy. *Journal of AOAC International*, **78**, No. 6, 1497-1501.

Pobozy, E., Wojasinska, E., and Trojanowicz, M (1996), Ion Chromatographic Specation of Chromium with Diphenylcabazide-based Spectrophotometric Detection. *Journal of Chromatography A*, **736**, 141-150.

Polansky, M. M., Bryden, N. A., Canary, J. J., and Anderson, R. A. (1990), Benefical Effects of Supplemental Chromium (Cr) on Glucose, Insulin and Glucagon of Subjects Consuming Controlled Low Chromium Diets. *FASEB J.*, **4**, 2964.

Powell, M. J., and Boomer, D. A. (1995), Determination of Chromium Species in Environmenal Samples using High Pressure Liquid Chromatography Direct Injection Nebulization and Inductively Coupled Plasma Mass Spectrometry. *Anal. Chem*, **87**, 2474-2478.

Rai, D., and Szelmeczka, W. (1990), Aqueous Behaviour of Chromium in Coal Fly Ash. *J. Environ. Qual.*, **19**, 378-382.

Rai, D., Eary, L. E. and Zachara, J. M. (1989), Environmental Chemistry of Chromium. Sci, *Total Environ*, **86**, 15-23.

Riley, T. and Watson, A. (1987), Polarography and Other Voltammetric Methods. Published by John Wiley and Sons Ltd..

Rubel, H. and Terytze, K. (1999), Determination of Extractable Chromium (VI) in Soils Using A Photometric Method. *Chemosphere*, Col. **39**, No. 4, 697-708,.

Sahayan, A., C., Arunachalan, J., and Gangadharan, S. (1998), Determination of Cr(VI) in Potable Water after Selective Separation of Cr(III) using ZnO. *Canadian Journal of Analytical Sciences and Spectroscopy*. **43**, No. 1, 4-7.

Samitz, M. H., and Katz, S. A. (1964), A Study of the Chemical Reactions between Chromium and Skin. *J. Invest. Dermatol.*, **43**, 35-43.

Saverwyns, S., Van Heck, K., Vanhaecke, F., Moens, L., and Dams, R. (1999), Evaulation of a Commerically available Microbore Anion Exchange Column for Chromium Speciation with Detction by ICP-Mass Spectrometry and Hyphenation with Microconcentric Nebulization. *Fresenius J. Anal Chem.* **363**:490-494.

Sirinawin. W., and Westerland, S. (1997), Analysis and Storage of Samples for Chromium Determination in Seawater. *Analytica Chimica Acta*, **356**, 35-40.

Standard Methods for Examination of Water and Wastewater. (1971), 13th Edition. Prepared and Published Jointly by American Public Health Association, American Water Works Association, and Water Pollution Control Association.

Toxicological Profile for Chromium, Draft, U. S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Refistry. October 1991, 43.

Tuman, R. W. (1978), Chromium Occurrence and Function in Biological Systems. *Physiol. Rev.*, **49**, 163-239.

Ultra-Violet and Visible Spectroscopy, (1967). Chemical Applications. 2nd Edition. London: Butterworths.

Urone, P. F., Anders, H. K., I. Bid. (1950), **22**, 1317.

Vukomanovic, D. V., vanLoon, G. W., Nakatsu, K., and Zoutman, D., E. (1997), Determination of Chromium (VI) and (III) by Adsorptive Stripping Voltammetry with Pyrocatechol Violet. *Microchemical Journal.* **57**, 86-95.

Wallach, S. (1990), Chromuim and Aging in Metal Ions in Biology and Medicine. P. Ph. Collary, L. A. Poirie, M, Manfait, and J. C. Etienne, Eds., John Libbey Eurotext, Paris, 299-303.

Walley, C., Hursthouse, A., Rowlett, S., Iqual-Zahid, P., Vaughan, H., and Durant, R. (1999), Chromium Speciation in Natural Waters Draining Contaminated Land. Glasgow, U.K. *Water, Air and Soil Pollution.* **112**:389-405.

Wang, J., and Lu, J. (December 1992), Measurement of Ultratrace Levels of Chromium by Adsorptive-Catalytic Stripping Voltammetry in the Presence of Cupferron. *Analyst*, **117**, 1913-1917.

Wang, J., Lu, J., Luo, D., Wang, J., and Tian, B. (1997), Simultaneous Adsorptive Stripping Voltammetric Measurements of Trace Chromium, Uranium, and Iron in the Presence of Cupferron. *Electroanalysis*, **9**, No. 6., 1247-1251.

Weber, H. (1983), Long-term Study of the Distribution of Soluble Chromate-51 in the Rat afte a Single Intra-trachael Administration. *J. Toxicol. Environ. Health*, **11**, 749-764.

Zarebski, J. (1977), Alternating Current, Normal and Differential Pulse Polarographic Studies of Chromium EDTA, CDTA and DTPA Complexes for Application to the Determination of Chromium in Trace Amounts. *Chemica Analityczna.* **22**, 1037.