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# ENVIRONMENTAL FATE AND EFFECTS OF CHROMIUM(III) AND (VI) INVESTIGATED USING ELECTROANALYTICAL CHEMISTRY

by

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*We start off confused, and end up confused, only at a higher level.*

## ABSTRACT

# ENVIRONMENTAL FATE AND EFFECTS OF CHROMIUM(III) and (VI) INVESTIGATED USING ELECTROANALYTICAL CHEMISTRY

**MICHAEL HENRY IRVIN COMBER**

A system which enabled the simple exchange of reagents during the electrochemical determination of chromium(III) or (VI) was developed. This media exchange system was used to investigate the effect of pH on the complexation and pre-concentration of chromium separately from the production of the electrochemical signal. While the complexation and preconcentration of chromium in fresh or sea water was slightly enhanced at pH 5, compared with pH 6.2, the electrochemical signal was markedly increased, being up to 10 times higher at the lower pH.

The addition of a column containing basic alumina, into the media exchange system, led to the development of a method for the selective determination of chromium(VI) and total chromium. The system had a detection limit of less than 1 $\mu g\ l^{-1}$, and was capable of determining chromium(VI) and total chromium in a sample in twenty minutes. The system was used on-board a boat to monitor the chromium levels in the Tees estuary. In this exercise, an unidentified interferent prevented levels of chromium from being measured. However, a procedure for overcoming this was developed, and an approach for investigating the contaminant investigated.

Using electrochemical methods for the determination of chromium(III) and (VI), the toxicity of these chromium oxidation states to three aquatic organisms, at varying salinities have been determined. A further study, explored the impact of organic chelators on the toxicity of both chromium oxidation states to one of the organisms, *Tisbe batagliai*.

The experiments demonstrated that chromium(III) was initially more toxic than chromium(VI). However, with increasing time the toxicity of chromium(VI) increased over that of chromium(III). It was also shown that at increased salinity there was a reduction in the toxicity of chromium(III). The effect of the organic ligands, EDTA, NTA and citric acid, was to reduce substantially the toxicity of chromium(III), but enhance that of chromium(VI) to *Tisbe*.

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## DEFINITION OF TERMS

**Speciation** - defined as "the process of identifying and quantifying the trace element forms present in a sample".

**LC50** - the concentration of a substance that kills 50% of the population exposed to that concentration.

**Environmental Quality Standard (EQS)** - An environmental quality standard is the highest concentration of a material that is acceptable in the receiving environment, immediately outside of an initial mixing zone of a discharge. It will have been derived from a mixture of experimental data from laboratory toxicity tests together with data from field observations.

**pH** - negative log of the hydrogen ion concentration of a solution

**pE** - redox potential - energy (in volts) gained in the transfer of one mole of electrons from an oxidant to hydrogen

**Ionic strength, I** - a measure of the interionic effects resulting from electrical attraction and repulsions between ions in solution. It is defined by

$I = 0.5 \times \text{sum}(C_i \times Z_i^2)$

for all C species with charge Z.

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## AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

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Adsorptive stripping voltammetry - a versatile technique, Loughborough, Nov 1987

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# CHAPTER 1.....INTRODUCTION

## 1.1 CHROMIUM SOURCES & USES

Although nearly 40 chromium minerals are known, (1), the only one used for production purposes, is chromite ore, $M^1O.M^2_2O_3$, where $M^1$ may be Mg or Fe, and $M^2$ may be Al, Fe or Cr. There are three major deposits of chromite, accounting for over 90% of the world's identified resources, these are located in South Africa, Zimbabwe and Finland. In 1980 the annual production of chromite was around 9.5 million tonnes/year.

Chromite ore is processed in one of two ways, depending on the intended use. It may be roasted with lime and soda ash, and then reacted with sulphuric acid to produce the dichromate. This is then treated electrochemically to obtain the pure metal. Alternatively, reduction of the ore with aluminium, silicon or carbon, produces ferrochromium. This may then be used in this form, for example in refractory bricks, or further treated by electrolysis to produce the metal.

Chromium has found a wide variety of uses, and these may dictate the initial form in which the metal will be released to the environment. The major uses of chromium with the predominant oxidation state, are given in table 1, (2). It is clear from this table, that the major uses

**Table 1 Main uses for chromium, and the oxidation state in which it is used.**

| USE | Description | % Use | Oxid. State |
|---|---|---|---|
| Steels | Added to iron to alter its properties, increases passivity, and depending on level, increases capacity to harden, and improves strength. | 61 | (VI) |
| Refractory materials | As chromite, used in manufacture of bricks, especially for furnaces. | 18 | (VI) |
| Pigments | Either as coloured, (lead chromate), or anti-corrosion, (zinc chromate) | 5.4 | (VI) |
| Metal finishing | Mainly for corrosion resistance, or visual enhancement. Frequently by electro-plating. | 4.4 | metal & (VI) |
| Leather tanning | chromium tanning, to increase resistance to bacteria, and improve its stability. | 3.3 | (III) |
| Water treatment | Added as a fungicide, and for its anti-corrosion properties. | 1.3 | (VI) |
| Wood treatment | With copper sulphate as a wood preservative, added as a fungicide. | 1.3 | (VI) |
| Catalysts | Especially in the petroleum industry. | 1.3 | (III) & (VI) |

of chromium are as the chromium(VI) ion. However, this does not necessarily mean that the major releases of chromium will follow the same trend. In a report issued by the American Petroleum Institute, (3), it was estimated that 80% of chromium being discharged into rivers came from either metal finishing plants, or sewage treatment works. This was supported by a 1984 report, (4), which claimed the main chromium(VI) inputs to rivers etc., included metal finishers, as well as cooling tower

effluents, and pigment production plants. The textile colouring industry and leather tanning works, were thought to be the major sources of chromium(III). However, the same report was not able to comment on the impact of sewage works on these chromium oxidation states.

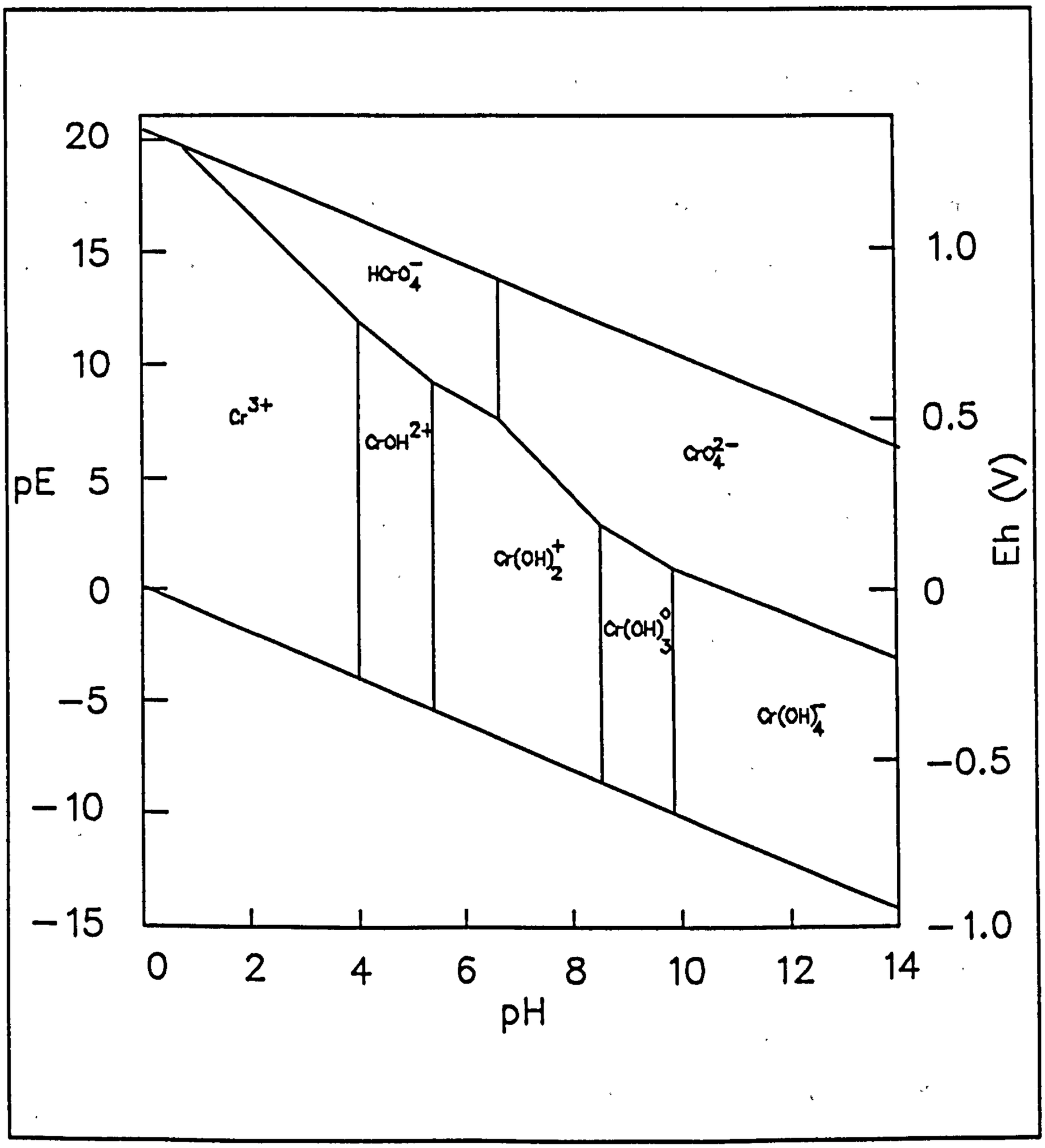
## 1.2 CHROMIUM CHEMISTRY

To understand the fate and effects of chromium, it is necessary to appreciate the chemistry of the oxidation states. The main forms, at environmental concentrations, are chromium(III), and chromium(VI), and the dominance of different forms of these oxidation states, depends on the pH, and the redox potential, pE, figure 1, (5).

### 1.2.1 Chromium(III)

The behaviour of chromium(III) is dominated by the formation of octahedral co-ordination complexes, characterised by very low reaction rates. Strong inert complexes are formed with a wide range of organic and inorganic ligands (6). This will lead to underestimation of chromium(III) in determinations based on complexation, depending on the stability constant of the complex formed. Thus Nakayama *et al.* showed that while amino acids had no effect on the recovery of chromium(III) in a co-precipitation method, malic acid, citric acid and ascorbic acid all led to reduced recoveries, in the pH range of 7 - 10, (7). In water, chromium(III) is

**Figure 1 Major forms of chromium(III) and chromium(VI) at 25°C and 1 atm. in water (5)**




gradually complexed by water molecules and eventually forms $Cr[(H_2O)_6]^{3+}$, (8), an extremely inert complex thus:

$$CrCl_3 \longrightarrow [Cr(H_2O)_4Cl_2]^+ + Cl^- \xrightarrow{\text{v.slow}} [Cr(H_2O)_6]^{3+} + 2Cl^-$$

Although very inert the complex does hydrolyse, thus:

$$[Cr(H_2O)_6]^{3+} \quad ====> \quad [Cr(H_2O)_5OH]^{2+} \quad + \quad H^+$$

The pK of this first hydrolysis product is 4, and as pH increases to environmentally relevant levels, the proportion of OH groups increases. The rate of change is, however, very slow, taking up to two months to reach equilibrium, (9).

The solubility of chromium(III) is complicated by this formation of a range of hydrolysis products, and also by the potential for organic complexation. This was demonstrated by James and Bartlett, (10), who showed that uncomplexed chromium(III) precipitated at pH higher than 5.5 - 6.0, but that when sodium citrate was added, the complex remained in solution up to pH 7.0 - 7.5.

This effect of organic complexation was also shown to effect the adsorption of chromium(III) to particulate matter by Musić, (11). Thus while chromium(III) was adsorbed with 100% efficiency at pH 6.5 and above, when sodium citrate was present, the adsorption was reduced as pH was raised above 6.5. This was further demonstrated when the rapid loss of chromium from river and seawaters occurred, (12), although this data needs to be interpreted carefully, as speciation analysis was not carried out.

The environmental behaviour of chromium(III) will depend,

therefore, on its low solubility in the pH range of 6 to 8, and its tendency to adsorb to particles. However, as discussed, the effect of organic complexation on this behaviour must also be taken into account.

### 1.2.2 Chromium(VI)

Chromium(VI) exists in one of two forms, with the chromate ion dimerising to dichromate at concentrations above $10^{-2}$ M. This concentration is too high to be encountered in the environment, so dichromate is unlikely to be present at significant concentrations, (13).

Chromium(VI) is a strong oxidising agent, and the chromate ion, figure 1, will exist either as $HCrO_4^-$ or $CrO_4^-$. Both forms are extremely soluble in water, and chromium(VI) is thus likely to be very mobile in the aquatic environment. Like chromium(III), it will adsorb onto particulates, although this declines as pH increases, due to the reduction in the positive surface charge, (11,14). Thus, for example, at pH 8, adsorption by iron oxides, alumina, or clay particles, was less than 30%, (15). However, in laboratory experiments the rate of achieving this equilibrium was found to be up to 3 days, (16), suggesting that in natural waters this behaviour may not be important.

### 1.2.3 Chromium transformation processes

a) chromium(III) to chromium(VI)

The principal mechanism for change from chromium(III) to chromium(VI) is based on the redox equilibrium;

$$Cr(H_2O)_4(OH)_2^{+} \quad ====> \quad CrO_4^{2-} + 6H^{+} + 2H_2O + 3e$$

In oxic waters, with a pH of 8.1 and pE of 12.5, this relationship predicts that chromium(VI) will be the predominant form of chromium, (17).

In the absence of other chemicals, the oxidation of chromium(III), does not occur rapidly. For example, Shroeder *et al.*, (18), found that at room temperature, in well oxygenated water, 3% of chromium(III) was converted to chromium(VI) after 30 days. However, the presence of $MnO_2$ led to increased reaction rates, whilst $Ca^{2+}$ and $Mg^{2+}$ inhibited the process. This has been further investigated by Fendorf & Zasoski, (19), who measured the electrophoretic mobility of the manganese particles as a function of surface charge. This was reduced as chromium(III) was oxidised, which they suggested was due to a surface alteration of the particles caused by surface precipitation. The need for manganese, to initiate the oxidation process was confirmed by Nakayama and co-workers, who also found that oxygen alone was insufficient, and that with complexation by citric acid

the oxidation was halted, (20).

These experiments suggest, therefore, that in estuarine regions, high in particulates, chromium(III) may be oxidised to chromium(VI). The transformation will, however, be limited by $Ca^{2+}$ and $Mg^{2+}$, organic complexation, as well as the time of contact with the particles.

b) chromium(VI) to chromium(III)

Chromium(VI) is a strong oxidising agent, and should be readily reduced to chromium(III) in the environment. However, reaction with dissolved organic matter does not appear to be a particularly important mechanism at environmental pH, especially in estuaries and seawater. Thus Eckert *et al.* demonstrated partial reduction, 94% at pH 2, 88% at pH 4, by fulvic acids, (21). This was confirmed by Nakayama and co-workers, with humics reducing chromium(VI) by less than 30% at pH 7, but nearly complete reduction at pH 4, (20). However, this will depend on the organic ligands present, as the same study found that chromium(VI) was completely reduced by ascorbic acid in the pH range of 7.7 - 8.2, after 72 hours.

An alternative reduction mechanism was investigated by Saleh *et al.*, (22), who showed chromium(VI) could be rapidly reduced by Fe(II) at pH 7, the reaction being:

$$3Fe^{2+} + CrO_4^{2-} + 7H_2O \longrightarrow 3Fe(OH)_3 + Cr(OH)_2^{+} + 3H^{+} .$$

A possible route producing Fe(II) from the photoreduction of Fe(III) particles with the oxidation of associated organic matter, (23), has also been described. This process was sufficiently fast, that the ratio of chromium(VI) : chromium(III) was reduced during the monitoring of a shallow estuary in daylight hours.

Another mechanism has been described by Shroeder *et al.*, (18), who studied the reduction of chromium(VI) in the presence of sulphides, present in anoxic environments. This was also a very rapid reaction, with the result that in their experiment over 50% of chromium(VI) had been reduced within 5 minutes of starting the experiment, and complete conversion occurred with 27 hours.

From the above it may be seen that there is potential for rapid transformation between the two oxidation states. Further, as pH changes, or the time from taking a sample to analysis increases, the possibility of exchange between chromium(III) and chromium(VI) will increase.

It is important therefore, that investigations aimed at understanding the environmental behaviour of chromium(III) and chromium(VI) must involve rapid analytical determinations, which are capable of discriminating between the two main chromium forms, as well as labile and bound chromium(III).

## 1.3 CHROMIUM ENVIRONMENTAL LEVELS

Although data on seawater has generally been confused, and not always in line with the accepted understanding of chromium speciation, more recent work has been more consistent. A number of studies have reported total chromium in seawater up to 5 $\mu g l^{-1}$, with most results below 0.5 $\mu g l^{-1}$. Chromium(III) represented 0 - 40% of that total, (24-27). There have been far fewer studies of estuarine regions, and the reported data so far does not appear to follow a consistent pattern. Thus Cranston and Murray, using a co-precipitation method, (28), described conservative mixing in the Columbia estuary, (29). This finding was contradicted by Campbell and Yeats, (30), who, while they only reported total chromium, showed that at the turbidity maximum there was a considerable reduction in dissolved chromium. Unfortunately, they did not measure pH, which means it is difficult to ascribe a reason for this behaviour. This highlights the need to measure a range of environmental descriptors when attempting to understand the behaviour of a metal, especially in the complex region of an estuary.

One attempt to try and quantify chromium(VI) and chromium(III) associated with negatively charged colloids was attempted by Hiraidi *et al.*, (31). The complexed chromium(III), with chromium(VI) was extracted onto an anion exchanger, DEAE-Sephadex A-25. Chromium(VI) was then reduced on column by hydroxylammonium chloride, and

eluted off, prior to determination. Chromium(III) was then eluted off the column by 4 M nitric acid. Although some river waters were analyzed, the potential for the method to be used on estuarine samples, with a higher ionic strength and particulate loading was not assessed. Neither was the level of recovery of chromium(VI) from the natural waters critically examined, a necessary step considering chromium(III) was being produced in the presence of the colloids on the column.

It is clear that little work has been carried out in estuarine waters, although this is essential, in order to understand fully the behaviour of chromium. This is an area of considerable complexity, with the potential for many organic interactions, and a high level of particulate concentrations. There are further complications caused by the ionic strength gradient that exists in the estuary, ranging from fresh to seawater.

## 1.4 BIOCHEMICAL INTERACTIONS

Chromium(III) is considered to be an essential trace element forming part of the Glucose Tolerance Factor, (32). It is postulated that as a co-factor of insulin it is part of the metabolism of glucose. It has generally found to be non-toxic to animals, and experiments considering the potential for cytotoxicity, have also demonstrated minimal effects, (33), except in the cell, when interstrand DNA crosslinks and DNA-protein cross-

linking occurs.

Chromium(VI), however, is rapidly absorbed into cells where it oxidises cellular matter, causing extensive damage. In a number of instances, the damage within a cell by chromium(VI) is the same as that caused by chromium(III). This would appear to suggest that the toxic agent is chromium(III), but that due to its immobility in biological systems, it can not exert the effect. Chromium(VI), however, is mobile, and once in the cell, or nucleus, exerts a toxic effect.

The data available for fish, and other aquatic organisms, reveals a confused situation. This may be due to the lack of speciation analysis carried out during such studies, and also to the impact of environmental factors on the complexation, and availability of chromium. These include the oxidation state, water pH, hardness, salinity, and the type and age of organism being tested. There is also the possibility, for chromium(VI), that the form of salt, *i.e.* chromate or dichromate is important, (34). This may be due to the speed of transfer across the cell boundary, or to differences in reactivity of the two salts.

A further parameter which also needs consideration is the type of study, *i.e.* was it short or long term, static or flow-through? It is possible that due to the differing mobilities of the two oxidation states, there could be a changing toxicity pattern during the test exposure.

Table 2 summarises some of the data available, which shows that there is a trend for chromium(III) to be more toxic in fresh, or uncomplexing media, and for chromium(VI) to be more toxic in seawater, or more complexing water. It is also noticeable that little comparative work has been carried out in seawater.

The effect of complexation on the toxicity of chromium was illustrated by two separate studies, looking at the impact of humic acid and ascorbic acid on chromium(III) and (VI) respectively. In the former, the addition of 5 $mgl^{-1}$ humic acid was sufficient to significantly reduce the toxicity of chromium(III) to *Daphnia pulex* by 72 and 96 hr, (35). A second study demonstrated the reduction of the toxicity of chromium(VI), this time to *Nostoc muscorum*, a cyanobacteria, by the addition of ascorbic acid, (36). While the reason for the former effect was likely to be due to reduced availability, in the case of the second experiment, the reason was put down to the reducing power of ascorbic acid, thus protecting the -SH group in amino acids. It is also possible that the ascorbic acid reduced the chromium in the extra-cellular area, but as no specific analyses were carried out, this could not be tested.

**Table 2 Comparative toxicity of chromium in water (Data in brackets are reported in this study, other data taken from reference 3)**

| Species | Cr(III) mg/l | Cr(VI) mg/l | Comments |
|---|---|---|---|
| FRESHWATER | | | |
| *Carrassius auratus* | 4.1 | 37.5 | 96 hr LC50 |
| *Leponius macrochirus* | 7.5 | 118 | 96 hr LC50<br>Soft water |
| | 75 | 133 | 96 hr LC50<br>Hard water |
| *Pimephales promelas* | 0.75 | 0.018 | Cr(III) - reduced wt @ 3 month<br><br>Cr(VI) - reduced wt @ 9 weeks |
| | 5.1 | 17.6 | 96 hr LC50<br>Soft water |
| | 67.4 | 27.3 | 96 hr LC50<br>Hard water |
| SEAWATER | | | |
| *Aldrichetta forstari* | 53 | 24 | 96 hr LC50 |
| *Crangon crangon* | (45) | (40) | 96 hr LC50 @ 30 ppt salinity |
| | (28) | (34) | 96 hr LC50 @ 20 ppt salinity |
| *Cyprinodon variegatus* | (45)<br>(45)<br>(45) | (80)<br>(40)<br>(28) | Median lethal conc.<br>@ 1 day<br>@ 3 days<br>@ 7 days |
| *Tisbe battagliai* | (0.2) | (1.4) | 48 hr LC50 @ 30 ppt salinity |
| | (0.2) | (1.2) | 48 hr LC50 @ 20 ppt salinity |

## 1.5 ANALYTICAL TECHNIQUES FOR CHROMIUM SPECIATION

The various analytical approaches used to measure chromium(III)/chromium(VI) concentrations fall into one of two groups, either physical separation *e.g.* co-precipitation, followed by detection, or direct detection using a differential analytical response, *e.g.* spectrophotometry or electro-chemistry.

### 1.5.1 Complexation

The range of complexing agents used in the determination of chromium(III) and chromium(VI) has been reviewed, (37). A typical approach is to first extract the complex of one oxidation state, convert the other oxidation state to the first, and repeat the analysis. Thus chromium(VI) has been extracted into methyl isobutyl ketone, (MIBK), after complexation with ammonium pyrrolidine dithiocarbamate, (APDC), at pH 3-4, chromium(III) was oxidized to chromium(VI) by addition of potassium permanganate, and the process repeated, (38). This procedure should be treated with caution, however, as the reduction of chromate at low pH by naturally occurring reducing agents has been reported, (20,39). The simultaneous extraction of chromium(III) and (VI) after complexation with APDC has also been reported, although the presence of humic acids, presumably complexing chromium(III), was reported to interfere with this approach, (40).

Another approach was based on the complexation of chromium(VI) by diphenylcabazide, (41), although, as this also needed to be done at low pH, the possibility of chromium(VI) being reduced to chromium(III) must be considered, (39).

The complexation of chromium(III) by quinolin-8-ol which may then be extracted, or collected on a polystyrene-divinylbenzene resin, (27), has also been used. The work reported examined the potential interfering effect of organic ligands and reducing agents, but it was noted that the levels considered were only appropriate for oceanic waters, and not for estuarine regions where the concentration of organic materials will be higher.

### 1.5.2 Co-precipitation

This is a popular technique frequently used as a multi-element method. Iron(II) has been used as a reagent a number of times, and co-precipitates with chromium(III) at pH 8, (28), chromium(VI) was also determined after reduction to chromium(III), the reported detection limit being 0.007 $\mu g l^{-1}$. A further use of co-precipitation was described where the carrier was again Fe(II), but this time after filtration the precipitate was analyzed directly by XRF, for chromium(III), (42). The pH of the filtrate was then adjusted to 4, APDC and cobalt added, forming an insoluble Co-APDC complex which co-precipitated the Cr(VI)-APDC complex. This was then filtered and also

analyzed by XRF. Using this technique the reported detection limit was 0.1 $\mu g l^{-1}$.

An alternative approach, with lead pyrrolidine dithiocarbamate has also been reported, (43). Chromium(VI) was extracted at pH 4, and then chromium(III) at pH 9. However, although the recovery of chromium(III) and (VI) was quantitative, no account for organic ligands was made.

A problem from which this approach suffers is the necessity to minimise contamination from the carrier, in this case iron salts. This generally leads to the need for very expensive ultra-pure carriers, and long and complex extraction techniques. A further problem has already been discussed above, 1.2.3.(b), that is the reactivity of ferrous ions with chromium(VI).

### 1.5.3 Ion exchange resins

Samples have been analyzed for total chromium using a spectrophotometric method, (44), and duplicate samples added to an anion exchange resin removing most of the chromium(VI). In this way chromium(III) was quantified directly and chromium(VI) by difference. However, not all the chromium(VI) was removed and a correction to account for the residual chromium was needed. Another application used the resin, polyethylene dithiocarbamate, (45). The

sample was passed through a column of the resin which was then digested and quantified by AAS. Total chromium, and by difference chromium(III), was quantified after oxidation of the sample by potassium permanganate and passage through another column of the resin. While the actual detection limit would depend on the quantity of water passed through the column, the reported limits were 12-36 $ugl^{-1}$.

The use of anion and cation exchangers has also been described, and applied to lakes, (46). However, the technique may again be criticised through the lack of measurement of total chromium, and thus colloidal chromium(III), (31).

An extremely sensitive method has been described, (47), in which chromium(VI) formed a complex with diphenyl carbazide adsorbed on a resin column in a flow cell. The absorption of light, at 550 nm, passed through the cell, was proportional to the quantity of complex absorbed on the resin. Although the method had a very low detection limit, <1 $ugl^{-1}$, and there was no interference from chromium(III), the cell needed careful calibration to account for the background shifts and attenuations that occurred.

### 1.5.4 Chromatography

Chromatographic separations are generally based on

complexation of chromium, followed by ultra-violet detection. Thus chromium-diethyl dithiocarbamate, (DDTC), complexes have been separated by reverse-phase high performance liquid chromatography, (RP-HPLC), (48). The analysis was performed twice, at pH 4, when only chromium(VI) reacted, and at pH 7 for total chromium. This was necessary as some chromium(III) was formed by the reaction of DDTC with chromium(VI), thus contributing to the chromium(III)-DDTC peak. Although the analysis time was short, less than 10 minutes, the samples were stood for 24 hours prior to analysis. As part of a multi-element separation DDTC was used to complex Cu, Ni, chromium(III) and chromium(VI), (49), using an electro-chemical detector with a glassy carbon electrode, in oxidation mode, at +1.1 V. Chromium(VI) again formed some chromium(III)-DDTC, but the amount was reproducible, and proportional to chromium(VI) concentration, so a correction could be applied.

Ethylenediaminetetraacetic acid, EDTA, has been used to complex chromium(III), either pre-injection, (50), when the samples were heated at 50°C, or on-column, (51), in the eluent. In both cases the analysis times were long, (up to thirty minutes), and other metals interfered by co-eluting with the chromium(III) and chromium(VI). Ion-pair RP-HPLC has been used after the samples were boiled with 4-(2-Pyridylazo) resorcinol, (PAR), and triethanolamine, (TEA). The TEA was added to accelerate the chromium(III)-PAR complexation, (52). The complex

eluted in five to six minutes but was followed by other metal chelates of PAR, and finally PAR itself. The pH of the eluent needed to be carefully controlled for optimum separation. An alternative ion-pair used has been by the addition of tetrabutylammonium phosphate to complex chromium(VI), (53). The method was used to investigate the breakdown of chromium(VI) in pond water. However, the method's limits of detection were 40 and 80 $\mu g l^{-1}$ for chromium(III) and (VI) respectively, and chromium(III) eluted in the void volume, which in real samples, with higher levels of organic materials, might lead to interferences.

An alternative approach to complexation-HPLC is ion chromatography, which has also been used for chromium(VI), (54), or for both chromium(III) and (VI), but not simultaneously, (55). The former method used an anion-exchange column, and the eluent was based on *p*-hydroxybenzoic acid, the concentration of PHBA and pH were optimised at 5 mM and 8.5 respectively. The detection limit, however, was too high for anything other than elevated levels of chromium, being 90 $\mu g l^{-1}$. The latter method investigated the potential of an anionic-exchange column with EDTA complexing chromium(III), and probably due to the lower charge density, eluted away from the void volume. However, the Cr-EDTA complex yielded a negative peak, leading to difficult interpretation, and the eluent was altered to one based on a borate buffer to allow for the elution of chromium(VI). In both cases the

detection limits were again too high for environmental samples. It is possible that linking such columns to an ICP-MS would considerably improve the detection limits and allow for the analyses of environmental samples.

### 1.5.5 Spectroscopy

There have been several studies looking at chromium(VI) only, which while they are likely to be selective, and sensitive, will need to include a chromium(III) to (VI) conversion step for a total chromium determination. In one example, Shaopu and Fuchang looked at the oxidation of iodide to tri-iodide, by chromium(III), and its subsequent complexation by Rhodamine B in the presence of poly(vinyl alcohol), (56). Unfortunately a number of environmentally important ions interfered with the determination and although octylamine was used to give extra sensitivity and selectivity, the time for analysis was lengthened, as the technique involved reduction of the sample size by heating. This would make it inapplicable for estuarine samples, containing a very high level of potentially interfering ions.

The use of PAR as a complexing agent in chromatographic methods was probably prompted by its use as a complexing agent in spectrophotometry. Samples refluxed with PAR for approximately 2 hours, have been quantified on the basis of an increased absorbance at 530nm, after extraction into chloroform, (57). The interferences from Fe,Co and Ni

were overcome by the addition of cupferron. The method was very sensitive with a limit of detection of 0.2 $ugl^{-1}$, but suffered from the need to reduce chromium(VI) to (III) prior to total chromium determination. A development of this approach led to the use of 2-(5-Bromo-2-pyradylazo) -5-diethylaminophenol, 5Br-PADAP, (58), which complexed chromium(III), although the sample needed heating to accelerate the reaction. A number of techniques were developed enabling chromium(III) and chromium(VI) to be determined in the absence of the other, and a further variant which included the initial measurement of chromium(VI) using diphenyl carbazide, followed by destruction of that complex and a total chromium determination using 5Br-PADAP, (59). The ability to apply this method to environmental samples would be limited, due to the amount of time needed to carry out the analyses, it is certainly questionable whether the initial determination of chromium(VI) would not include organically bound chromium(III). The method will also suffer from the same problems mentioned below.

Diphenyl carbazide, (DPC), has been used for measuring chromium(VI), for example in animal digests, chromium(III) was oxidized using cerium(IV), and the total chromium complexed and measured by spectrophotometry, (41,60). The method suffered from interferences including phosphate and iron. The latter being extracted as the DPC complex into dichloromethane.

### 1.5.6 Electrochemical techniques

#### 1.5.6.1 General

(Note - all potentials are versus the Standard Calomel Electrode)

In an early review of chromium electrochemistry, (61), two half wave potentials are mentioned for the chromic ion in KCl, at a dropping mercury electrode:

@ -0.88 V, thought to be Cr(III) --> Cr(II), and

@ -1.53 V, probably Cr(II) --> $Cr^0$

It was also noted that complexation of chromium(III) moved the peak in a negative direction; thus chromic tartrate complex had a half-wave potential at -1.0 V. In a more recent investigation the same two-step process was again investigated, (62). The formation of $Cr^0$ was found to be irreversible, due to a fast reaction, which lead to the deactivation of $Cr^0$. This situation is complicated by the complexation of Cr(II) when EDTA is present, and re-oxidation to chromium(III), (63). Thus in the reduction of hexamminechromium(III), adding EDTA at low pH caused two peaks to appear, the first of which, (at -0.9 V), gradually disappeared as the EDTA concentration increased. This may be attributed to:

a) reduction of the hexamminechromium(III) at -0.9 V to Cr(II);

b) formation of a Cr(II)-EDTA complex followed by oxidation to Cr(III)-EDTA, provided the potential is positive enough;

c) reduction of the Cr(III)-EDTA complex to Cr(II) at -1.23V.

Table 3 summarises these general points for the electrochemical behaviour of chromium.

**Table 3 The electrochemical behaviour of chromium ions**

| OXIDATION STATE | POTENTIAL/V | COMMENTS |
|---|---|---|
| Cr(VI)-->Cr(III) | -0.3/-0.5 & -0.8/-1.0 | Depends on pH, and is affected by $Cr(OH)_3$ adsorption on the mercury drop. |
| Cr(III)-->Cr(II) | -0.8/-1.3 | Complexation pushes the peak in a cathodic direction. |
| Cr(II)-->$Cr^0$ | -1.5/-1.7 | |

### 1.5.6.2 Electroanalysis

Chromium(III) has not usually been determined directly, but an exception to this was noted by Kheifets *et al.*, (64), who described a number of peaks obtained from chromium(III) and chromium(VI) solutions in ammoniacal buffer, at pH 8-9. Of the peaks obtained for chromium(III) the response at -1.6 V, was used for analysis, after enhancement by addition of barium chloride, to bind any chromium(VI), and nitrate ions, to catalyse the chromium(III) reduction current. Although

not immediately applicable to environmental analyses, two points of interest did emerge from this study. Firstly the addition of barium chloride to remove chromium(VI) led to an improved chromium(III) peak shape. Secondly the peak assigned to chromium(III) was noted as disappearing in natural waters, although the same behaviour was not noted in distilled water.

Differential pulse polarography, (DPP), was used to determine directly chromium(VI), (65), when the addition of $F^-$, a strong complexing agent, was necessary to prevent the passivation of the electrode by chromium(III). Although detection limits of approximately $2x10^{-8}$ M chromium(VI) were obtained, as little as 1 mM of chloride ions interfered with the chromium(VI) peak, at -0.2 V, and reduced the response. The presence of fluoride reduced the interference by copper, but in tartrate buffer systems the chromium response was significantly reduced. An alternative approach was based on the deposition of $Cr(OH)_3$ film on a glassy carbon electrode formed from chromium(VI) at potentials between -0.6 and -1.0 V, (66). This was followed by dissolution of the film by anodically scanning the electrode from 0.6 to 1.0 V producing an oxidation wave for chromium(III) to chromium(VI). Although calibration curves down to $1x10^{-6}$ M chromium(VI) were obtained, the responses needed careful interpretation, being variable due to the irreversibility of the chromium(III)/chromium(VI) redox system.

The reduction of chromium(VI) in the electrochemical cell to chromium(III) followed by complexation, preconcentration and then electrochemical stripping has been investigated. Thus in ammonium chloride buffer at pH 9.3, a dimethyl glyoxime, (DMG), complex was formed with chromium(III) yielding a peak at -1.45 V, (67).

The suggested mechanism was :

$$CrO_4^- + 4H_2O + 3e \Longrightarrow Cr(OH)_3 + 5OH^-$$

and

$$Cr(OH)_3 + 6NH_3 \Longrightarrow [Cr(NH)_6](OH)_3$$

which then complexed with the DMG. The reduction of the chromium(III) was a one electron step resulting in desorbed DMG and $Cr(NH_3)^{4+}$.

Malakhova *et al.* used the complexation of chromium(VI) by 1,5 diphenylcarbazide in the determination of chromium in environmental samples, (68). An advantage of this approach was that as oxygen did not affect the peak shape or height, the samples did not need degassing. However, no attempt was made to investigate the recovery of chromium(VI) in environmental samples, which at the low pH used could well have been reduced. The method was investigated further, (69), when the effect of fulvic acids was found to reduce the recovery of chromium(VI) by up to 40%. The method described could only be used for chromium(VI), however, as UV irradiation to oxidise

chromium(III) rendered the sample unusable due to secondary reactions.

Finally a method based on the complexation of chromium(III) by diethylenetriaminepentaacetic acid, (DTPA) has been used for a wide variety of samples. Thus chromium(III) signals were found to be stable in the absence of nitrate, while the response to chromium(III) and (VI) was similar, (25,70). In the latter when nitrate was added, 2 peaks were initially observed which were assigned to an unknown product of the catalytic oxidation of chromium(II)-DTPA, thus :

$$Cr(II)\text{-}DTPA^{-} + NO_3^{-} \;\;\text{====>}\;\; Cr(III)\text{-}DTPA^{-} + X$$

In the work reported by Boussemart *et al.*, (25), the method was used for the determination of chromium in seawater. The principal problem, however, was that total chromium was determined, and chromium(III) and (VI) estimated by the reduction in the electrochemical signal after 30 minutes. This reduction was due to Cr(III) loss, the stable portion of the signal being assigned to chromium(VI).

In other studies, (71,72), samples were first treated to oxidize all the chromium to chromium(VI), which subsequently reduced to chromium(III) at potentials less than -0.4 V, reacted with DTPA adsorbed to the mercury drop and was stripped at -1.22 V. As noted above, nitrate

was added to catalyse the response, which was optimal at pH 6.3. Under the conditions described the method had a detection limit of 50 $ngl^{-1}$. However, no attempt was made to speciate the chromium, only total concentrations being measured. The effect of hydrogen peroxide, used in the oxidation of chromium(III), was found to interfere with the subsequent analyses, (73), and an alternative procedure using oxygen saturated samples was proposed. The method was extended towards speciation by measuring chromium(VI) after precipitation of chromium(III) with aluminium hydroxide. This approach did affect the precision of the determination of chromium(VI) leading to recoveries of 70 - 120% at 100 $nmoll^{-1}$. In an investigation of these reactions in the presence of boric acid, some differences were noted, (74). First chromium(VI) yielded a peak 1.4 times that of chromium(III), and the response to chromium(III) was reduced by 15% after 5 minutes. Although pH, temperature, reagent concentrations and the order of their addition were investigated no obvious reason was found. The method was quite lengthy involving boiling with bromine water, to oxidize the chromium(III), removal of the excess bromine water and pH adjustments. However, the detection limit was approximately 0.02 $ugl^{-1}$ and the method was used for a number of environmental samples.

The importance of electroanalytical techniques in estimating the biological fraction has been exploited for other metals, (75-79). These have used the advantages of electro-chemistry being fast, sensitive, and easily

modified to determine different oxidation states, or vary the extent to which complexed metals will react in the electrochemical cell. All this suggests that electroanalysis is a powerful tool to be used in environmental investigations of chromium.

## 1.6 AIMS AND OBJECTIVES

The work was undertaken to fulfil the following aims.

1. To investigate the electroanalytical chemistry of chromium(III) and chromium(VI), particularly in the presence of each other and a variety of ligands.

2. To develop suitable electrochemical techniques, such as differential pulse anodic stripping voltammetry, (DPASV), and cathodic stripping voltammetry, (CSV), for the determination of chromium(III) and chromium(VI) in the presence of each other, and to develop these techniques further, to enable rapid analyses to be carried out.

3. To apply these methods to environmental studies, in estuarine areas, and, combined with other approaches, *e.g.* speciation modelling by computer, develop a better understanding of the form of chromium inputs to estuaries, and the behaviour of chromium discharged to such environments.

4. To investigate the potential of electroanalytical

techniques to aid in the determination of the bioavailable fraction of chromium.

In order to meet these aims, it was necessary that a number of objectives were achieved. These were defined as follows.

1. Investigate the CSV method based on the complexation of chromium(III) by DTPA, with particular interest being paid to chromium(VI) responses and the interactions in such a system between chromium(III) and chromium(VI). initially through the application of cyclic voltammetry, (CV).

2. To understand the electrochemistry involved by changing the electrochemical cell conditions. This would be achieved by adding different complexing agents, altering the buffer and the pH of the cell contents, and varying the CV scan parameters.

3. To investigate the potential for a speciation modelling program to aid in the understanding of the chemical processes involved in the electrochemical cell, the behaviour of chromium in estuarine environments and in aquatic toxicity experiments.

4. Develop electroanalytical methods for chromium(III) and chromium(VI) which would satisfy the aims above, being fast, sensitive, capable of being automated, and above

all, of high selectivity to the oxidation state being determined.

5. To use the methods developed to investigate, in situ, the behaviour of chromium in an estuary and to contrast this with its behaviour as predicted by the model.

6. To carry out aquatic toxicity tests to fish and other appropriate organisms, and to aid the understanding of the results using electroanalysis and modelling of chromium speciation.

# CHAPTER 2.....STABILITY MODELS & DEVELOPMENTS

## 2.1 INTRODUCTION

As described in section 1.4 the biological impact of chromium is due to the oxidation state present, and to the environmental factors, which will influence the extent of complexation of the chromium ions. This is true for most metals which have been studied, and an early review of trace metal speciation in general was given by Florence and Batley, (6). One result of these effects was an interest in the use of chemical modelling. This is because from a limited data set, it is possible to describe in some detail many situations that could not otherwise be investigated. It is also possible to predict the effect of varying inputs to a system *e.g.* metal concentrations, or the level and type of organic ligands.

The program used in this study was MINEQL, obtained from Professor F M M Morel, Massachusetts Institute of Technology. The program had been further developed by Dr D Cummins and Dr M Turpin, Unilever Research Centre, Port Sunlight. They had developed other blocks, to form an integrated package of speciation modelling programmes and techniques, called Chemical Equilibrium Simulation Guidance, CESG. As supplied, it was possible to add or change stability constants and explore the effect on chromium speciation of a variety of parameters. The program is described in more detail in section 2.2.4.

## 2.2 THEORY

### 2.2.1 General

When solving chemical equilibrium problems in multi-component systems, two sets of conditions need to be met. The first, mass balance, dictates that the total concentration of any component after computation of its various forms, must equal the initial concentration of that component. The second, is that the calculated chemical equilibria must equal the most stable configuration of the system, given constant mass balance.

There are two approaches to calculating the equilibrium of a system. If an initial estimate of a feasible solution to the mass balance equations is made, then subject to the constraints of the mass balance equations, the Gibbs Free Energy may be minimised. This approach was initially described by White *et al.*, (80). The second method was first described by Brinkley, (81,82). An initial set of equations for the components is made, using equilibrium constants. The mass balance equations are then solved by iteration. The program used in this study, MINEQL, is based on this latter method.

The choice between which approach to take is generally based on two specific requirements of the methods. Both require a data base, in the case of the former method, of free energy values, and in the latter, equilibrium

constants. It is frequently the case that equilibrium constants are more available, and easier to produce, than free energy constants. The second requirement relates to the mathematical approaches for solving the set of nonlinear equations that either method establishes. An iterative process is needed, as it is not feasible to solve a multicomponent, multiphase system simultaneously. When seeking to minimise the Gibbs Free Energy, the appropriate techniques are steepest descent, linear programming and gradient methods, all optimisation techniques. In the case of the equilibrium constant method, an approach described as the Newton-Raphson iterative approach, which includes nested iterations and successive approximations, is used.

### 2.2.2 Equilibration models

To consider how equilibrium models are developed and computed, the simple system of a metal with a ligand in water is modelled. For this exercise it is assumed that water does not interact with either component, that other ions present will interact, so the total concentration of metal, M, and ligand, L, need not be equal to balance charges, and non-ideal behaviour is not considered.

if $M^{++}$ forms complexes with $L^{-}$, thus;

$$M^{++} + L^{-} \text{ ==== } ML^{+} \qquad K_1 \qquad (1)$$

$$M^{++} + 2L^{-} \text{ ==== } ML_2^{0} \qquad K_2 \qquad (2)$$

$$M^{++} + 3L^{-} \text{ ==== } ML_3^{-} \qquad K_3 \qquad (3)$$

The formation constants are given from the relationships;

$$K_1 \quad === \quad [ML^+] \;/\; [M^{++}][L^-] \tag{4}$$

$$K_2 \quad === \quad [ML_2^0] \;/\; [M^{++}][L^-]^2 \tag{5}$$

$$K_3 \quad === \quad [ML_3^-] \;/\; [M^{++}][L^-]^3 \tag{6}$$

In a similar manner, the mass balances, for total metal, $M_T$, and total ligand, $L_T$, are;

$$[M_T] \quad = \quad [M^{++}] + [ML^+] + [ML_2^0] + [ML_3^-] \tag{7}$$

$$[L_T] \quad = \quad [L^-] + [ML^+] + 2[ML_2^0] + 3[ML_3^-] \tag{8}$$

Similar equations may then be set up for any components present as solids;

$$ML_2(s) \quad === \quad M^{++} + 2L^- \qquad K_s \tag{9}$$

and $K_s$, the solubility product, is

$$K_s \quad === \quad [M^{++}][L^-]^2 \tag{10}$$

The mass balance for each component is then modified to include the concentration of $ML_2(s)$.

By re-organising the equations defining the stability constants, the following general equations are obtained,

$$[C_{i,j,k}] \quad \equiv \quad K_{i,j,k}\,[M_i]^{\alpha}\,[L_j]^{\beta}\,[H]^{\gamma} \tag{11}$$

where,

$C_{i,j,k}$ = the *k*th complex of $M_i$ and $L_j$ , thus $M_i$–$L_j$

and

$H^{\gamma}$ = the stoichiometric coefficient of the hydrogen ion, when $\gamma$ is positive it represents $H^+$, and when negative it represents $OH^-$.

In practice, it is easier to solve the simultaneous equations for each metal and ligand, with a further equation for the proton, by rearranging the equations, such that the complexes are treated as secondary unknowns. In this way the equation for each metal and ligand may be represented thus;

$$TOTM_i = [M_i] + \sum_{j,k}\alpha_{i,j,k}\ [C_{i,j,k}] \qquad (12)$$

and

$$TOTL_j = [L_j] + \sum_{i,k}\beta_{i,j,k}\ [C_{i,j,k}] \qquad (13)$$

If the pH is not known, there needs to be a further equation defining *TOTH*. In the study being described, the pH was always either known, or a variable being investigated, thus calculating the concentration of the hydrogen ion was a trivial problem.

With equations 12 and 13 defining the complete system, the equations are then solved by the Newton-Raphson method. The mathematics of this are considered to be beyond the scope of this study, and so will not be described. However, van Zeggeren and Storey, (83), provided a good

summary of this approach.

### 2.2.3 Comments on interpretation of model predictions

There are a number of factors which need to be considered when interpreting the results from this modelling approach. The first problem relates to the quality of the data in the data base. If the reliability of, for example, the equilibrium constants used is uncertain, then so will be the results obtained. This is a concern with the equilibrium constant approach particularly, because there is considerable disagreement in the literature over the values to be used. Thus French, (84), in developing an equilibrium model for aluminium, was unable to use a "correct" set of constants. Instead the available constants were critically evaluated, and in at least one instance, two constants were used, French being unable to choose between them.

Further inaccuracies will be caused to the predictions if important complexes are not included in the calculations. This may be due to their existence not being suspected, or because there are no equilibrium constants available. This is one of the major drawbacks of this approach, and care must always be taken when assessing the results from the models. The two issues of prime importance in this study were those relating to organic interactions, *e.g.* complexation by humics, and adsorption by particulate matter.

The effect of temperature also needs to be considered, as in most cases models are based on data obtained at 25°C. This may cause, depending on the complexes considered, severe disturbances of the calculated equilibria. Thus Byrne *et al.*, (85), considered the effect of temperature on trace metal speciation in seawater. They found that metals whose complexation was dominated by hydrolysis, were strongly influenced by a temperature change of 5°C to 25°C, with the level of hydrolysed metal increasing. Generally, however, there is insufficient data to be able to predict accurately the effect of temperature on trace metal speciation in the environment.

A further complication of models, relates to the need to correct for the ionic strength of the media being modelled. This is because the data base of such models usually contains data which has been measured at low, <0.5M, ionic strengths, which are subsequently corrected to zero ionic strength. These are then corrected to the appropriate ionic strength of the test case, using either the Davies or Debye-Huckel corrections, (86).

*e.g.* the Davies correction:

$$\log\gamma_z = 0.5z^2 \left( \frac{I^{\frac{1}{2}}}{\left(1 + I^{\frac{1}{2}}\right)} - 0.3I \right)$$

where $\gamma$ = activity coefficient of species of charge z and I = ionic strength.

However, these are limited in the range of ionic strengths they are applicable to, with the Davies correction being favoured at ionic strengths up to 0.5. Above this the correction begins to fail, although it is still used, as the errors introduced are normally much smaller than those caused by inaccuracies in the determination of the equilibration constants being used, (87). This does mean, however, that care must be taken when modelling in seawater, which has an ionic strength of 0.7.

A problem which also needs to be considered in comparing data from models, or in applying the predictions, relates to assumptions made concerning the media being modelled. Examples of various "average" waters are shown in table 4. Although basically similar, there are some differences, *e.g.* the trace metal concentrations, and the pH of the river water. It is also clear from the table that organics were not included automatically, though they were investigated, and found to have a significant effect on the predicted trace metal forms likely to be present. Another complication which needs to considered, is that these methods are all equilibrium models. It is highly unlikely given the dynamic state of estuaries *etc.*, the variety of inputs, and in some cases the time required for a reaction to reach equilibrium, see section 1.2.1, that an equilibrium will be reached in most of the environment.

A final criticism concerns the assumptions and treatment of redox processes. In an assessment of the impact of

**Table 4 Examples of "average" waters used in modelling of trace metal speciation**

| | River Water (mM) (88) | River Water (mM) (89) | Seawater (mM) (88) | Seawater (mM) (89) |
|---|---|---|---|---|
| Na | 0.270 | 0.52 | 480 | 485 |
| K | 0.059 | 0.035 | 10 | 10.6 |
| Li | - | - | - | 0.027 |
| Ca | 0.370 | 0.30 | 10 | 10.7 |
| Mg | 0.170 | 0.31 | 50 | 55 |
| Cr | - | 0.00001 | - | 0.000006 |
| Cu | 0.00008 | 0.000008 | 0.000076 | 0.00001 |
| Pb | 0.000014 | 0.00000014 | 0.00000001 | 0.0000003 |
| Zn | 0.00015 | 0.000006 | 0.000076 | 0.00008 |
| Cd | 0.0000008 | 0.0000009 | 0.00000004 | 0.0000009 |
| Hg | 0.00000004 | 0.00000005 | 0.00000025 | 0.0000002 |
| Ni | 0.000005 | 0.000031 | 0.000034 | 0.00002 |
| Al | - | 0.00019 | - | 0.00008 |
| Fe(II) | - | 0.00027 | - | 0.00045 |
| Fe(III) | - | 0.000013 | - | 0.0017 |
| Cl | 0.22 | 0.28 | 560 | 566 |
| F | - | 0.005 | 0.06 | 0.08 |
| Br | - | 0.00008 | 1.0 | 0.87 |
| $SO_4$ | 0.12 | 0.08 | 12 | 29 |
| $PO_4$ | 0.0056 | 0.0022 | 0.00032 | 0.0007 |
| $NO_3$ | 0.016 | 0.014 | 0.0081 | 0.005 |
| $NH_4$ | - | 0.008 | - | 0.002 |
| Humics | - | - | - | - |
| pH | 6, 7, 8 | 8.01 | 8 | 8.22 |

several redox options on the Fe(II)/Fe(III) and As(III)/As(V) couples, ranges of concentrations differing in several orders of magnitude were obtained, (89). It was recommended these should be examined separately where such redox elements were an important part of the model.

In the following section, describing the programme used in this project, MINEQL, these objections will be further discussed, and attempts made to overcome them described.

### 2.2.4 MINEQL

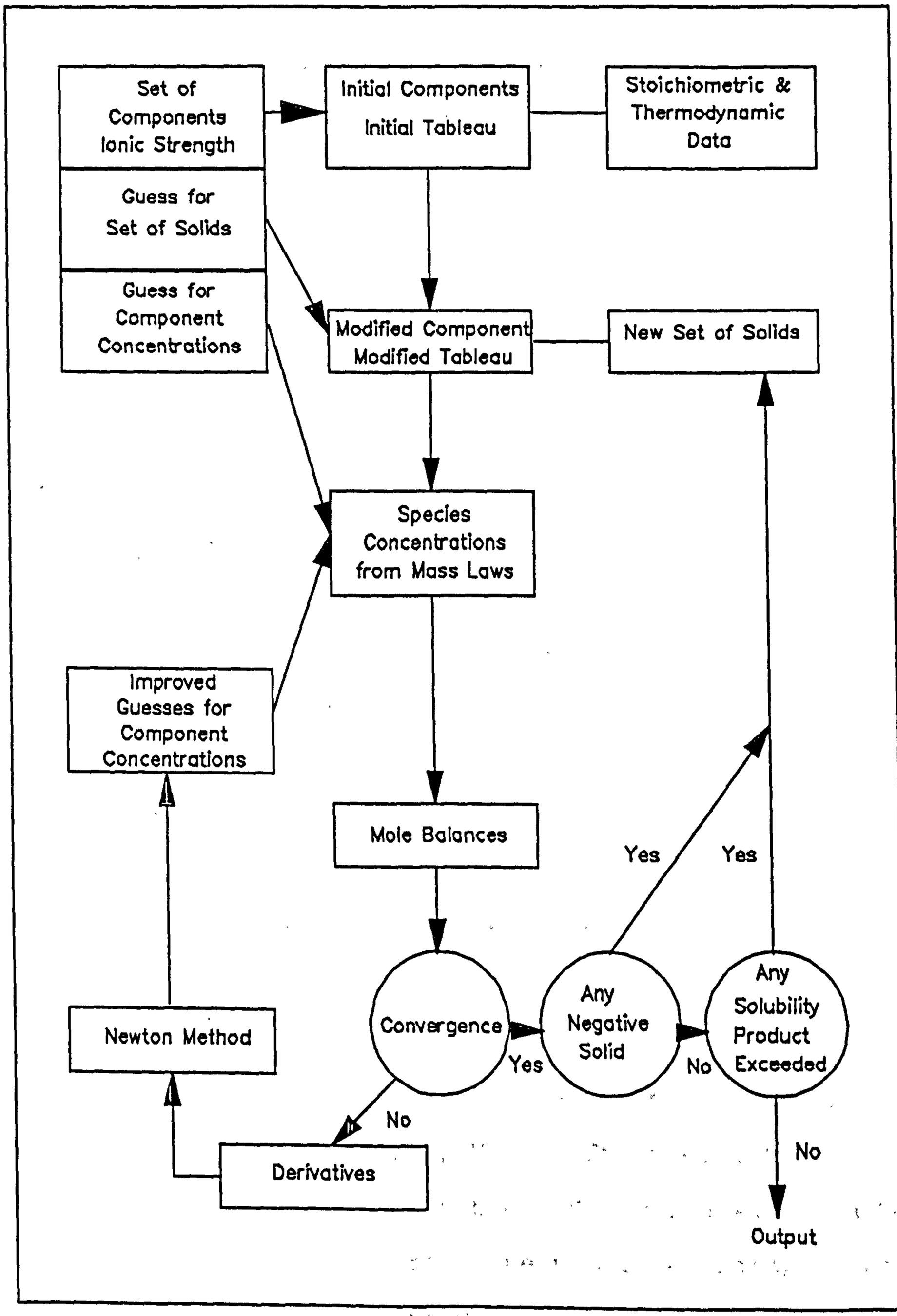
The program, MINEQL, was initially described by Morel and Morgan, (90), and subsequently a simple description of the algorithm of the program, figure 2, was published, (87). As may be seen from figure 2, the program also takes into account the production of solids, and their precipitation.

The program obtained, CESG, included a data base of formation constants, with references, including the experimental temperature and ionic strength at time of determination. The method used to correct to zero ionic strength, *e.g.* Davies, is also included, and an assessment of the reliability of the data has also been made.

As used in this study the formation constants data base had been further reviewed and additions made by the author; this version will be referred to as MINEQL-MHIC.

Within the program a discrimination is made between components, *e.g.* $Cu^{++}$, or $NO_3^-$, and species made up from these components, *e.g.* $Cu(NO_3)_2$. The initial set of components, with concentrations, is entered together with identification of the species for which a summary is needed.

**Figure 2 Algorithm for MINEQL, (87)**



The printout options available include:

a) all components and species formed,

b) specified components and/or species formed,

c) a summary of specified components, and the major species, as a % distribution.

An estimate of the initial ionic strength is made, based on the following:

$$0.5 \times \text{Sum of all sol. species } [\text{Conc} * \text{charge}^{\wedge 2}].$$

As mentioned above, changes in ionic strength, may lead to errors. The program was subsequently modified by D Cummings and M Turpin to perform recalculations of the ionic strength, depending on the formation of any precipitated solids, and new components in the system. The equilibrium constants are then recalculated, using the Davies equation, to take account of the new ionic strength. Although the Davies equation may introduce errors, these are generally regarded as being of minor importance.

The output from the model program was then extracted, and transferred to a spreadsheet, Microsoft Excel (c), Microsoft Corporation, Washington, USA, which was used to arrange the data in an appropriate tabulated form, or for transfer of the data to a program for generating figures.

For initial modelling purposes, and for filling in blank

values when modelling partially characterised solutions, the data used was that described by Nordstrom *et al.*, (89). This data was chosen for two reasons. Firstly, having been used for comparing several models, it enabled the program to be checked for consistency, and major discrepancies to be more easily identified. Secondly the data used was more extensive than that described by French, (88), which thus minimised the chances of missing important interactions.

When modelling an estuarine environment, the average river was assumed to mix conservatively with the seawater. The concentrations of the trace metals and other elements were then calculated for the intermediate compositions. The values of pH were based on the data from French as the estuary to be investigated, the Tees, was known to have a riverine input of about pH 6. The compartments modelled were 0‰, 2‰, 5‰, 10‰, 15‰, 20‰, 30‰ and 35‰ salinity.

To ensure particulate adsorption was taken into account, a value for the concentration of particulates was put into the model. This was assumed to have a major impact at the riverine input to the estuary, with a linear decrease in concentration with the increase in salinity. However, it must not be forgotten that in stratified rivers, like the Tees, there is frequently a saline wedge, with fresh water overlying seawater. In such systems, there is frequently a large concentration of particulates at the boundary of these two layers.

The equilibrium between chromium and particulate matter was based on the following reactions;

$$Cr(VI) + SOH \quad ====> \quad SOH\text{-}Cr(VI)$$

$$Cr(III) + SOH \quad ====> \quad SOH\text{-}Cr(III)$$

where SOH represent surface hydroxyl groups on particulate matter.

A review of the available data on concentrations of particulate matter, and associated equilibrium constants yielded very little data. Furthermore, because of the potential redox processes which may occur, the data that is available should be treated with caution. Movet and Bourg, (91) did estimate some equilibrium data for metals, and suggested a relationship with the hydrolysis constants of those metals investigated;

$$\log\beta_1^{surface} = 0.945\log\beta_1^{hydrolysis} + 5.6$$

Using this relationship an initial estimate for the equilibrium constant of Cr(III) - ADS was made, $K = 1.72$, where ADS represents the adsorbing particulate matter. Muvet also suggested that a value of 8M was a suitable estimate for the concentration of particulates in the river. This value was used as an initial estimate for the Tees.

A similar process was followed for the possible impact of

organics on chromium speciation, based on the following reactions;

$$Cr(VI) + OL \quad ====> \quad OL\text{-}Cr(VI)$$

$$Cr(III) + OL \quad ====> \quad OL\text{-}Cr(III)$$

where OL represents organic ligands.

Although there is an extensive data base of organic matter determinations available, data relating to the molecular weight of this matter is less easy to come by. Estimated concentrations for humics in rivers range from 0.1μM to over 400μM. This latter figure being suggested as a reasonable average for world river inputs to the sea, (92). As such inputs included some very large rivers, *e.g.* the Amazon, it is probable that the level contributed by rivers such as the Tees will be much less. The concentration taken is based on the data from Nordstrom, (88), modified where data was available for the Tees.

In order to assess the effect of a range of organic ligands, two potential organic chelators were chosen. These were citric acid as a weak binding ligand, and EDTA as a very strong ligand.

Finally to investigate the possibility of redox processes based on pE affecting chromium speciation, at least one model was run with the redox equation included;

$$Cr(H_2O)_4(OH)_2^+ \quad ====> \quad CrO_4^- \quad + \quad 6H^+ \quad + \quad 2H_2O \quad + 3e$$

Table 5 summarises the data used for the estuarine models, including the concentrations of particulate and organic matter.

## 2.3 VALIDATION EXERCISES

To ensure that the model being run in this study was consistent with previously reported models, it was decided to run the model initially as described by Nordstrom, (88). The data for the river, and seawater were used as shown in table 5.

A selected number of species were then compared, the results are shown in table 6.

### 2.3.1 Comparison of data from river water model

Care must be taken in comparing these data, for a number of reasons. First the programs being compared are not identical, the one used in this study, (MINEQL-MHIC), is an earlier version of that used by Morel in the exercise carried out by Nordstrom, (MINEQL-N). Secondly there will be differences in the stability data used, thus the data base in MINEQL-MHIC is very recent, updated in 1990. Finally the data is expressed as the log(molarity), thus the ratio expressed in table 6, must be considered along with the actual concentration being predicted.

**Table 5** Data used in initial investigations of model estuary for investigating chromium speciation (all concentration data in mMole, unless stated)

| | River | 2‰ | 5‰ | 10‰ | 15‰ | 20‰ | 30‰ | 35‰ |
|---|---|---|---|---|---|---|---|---|
| Na | 0.52 | 28 | 70 | 139 | 208 | 277 | 416 | 485 |
| K | 0.035 | 0.64 | 1.54 | 3.05 | 4.56 | 6.07 | 9.09 | 10.6 |
| Li | 0 | 0.0015 | 0.0039 | 0.0077 | 0.0116 | 0.015 | 0.023 | 0.027 |
| Ca | 0.30 | 0.89 | 1.786 | 3.27 | 4.76 | 6.24 | 9.21 | 10.7 |
| Mg | 0.31 | 3.44 | 8.12 | 15.9 | 23.7 | 31.6 | 47.2 | 55 |
| Cr* | 10 | 9.8 | 9.4 | 8.9 | 8.3 | 7.7 | 6.6 | 6 |
| Cu* | 8 | 8.1 | 8.3 | 8.6 | 8.9 | 9.1 | 9.7 | 10 |
| Pb* | 0.14 | 0.15 | 0.16 | 0.19 | 0.21 | 0.23 | 0.28 | 0.3 |
| Zn* | 6 | 11 | 17 | 28 | 38 | 49 | 70 | 80 |
| Cd* | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Hg* | 0.05 | 0.06 | 0.07 | 0.09 | 0.11 | 0.14 | 0.18 | 0.2 |
| Ni* | 31 | 30 | 29 | 28 | 26 | 25 | 22 | 20 |
| Al* | 190 | 184 | 174 | 159 | 143 | 127 | 96 | 80 |
| Fe2* | 270 | 280 | 296 | 321 | 347 | 373 | 424 | 450 |
| Fe3* | 13 | 109 | 254 | 495 | 736 | 977 | 1459 | 1700 |
| Cl | 0.28 | 33 | 81 | 162 | 243 | 324 | 485 | 566 |
| F | 0.005 | 0.009 | 0.0157 | 0.026 | 0.037 | 0.048 | 0.069 | 0.08 |
| Br | 0.00008 | 0.05 | 0.12 | 0.25 | 0.37 | 0.5 | 0.75 | 0.87 |
| $SO_4$ | 0.08 | 1.7 | 4.2 | 8.3 | 12.5 | 16.6 | 25 | 29 |
| $PO_4$* | 2200 | 2100 | 2000 | 1800 | 1600 | 1300 | 900 | 700 |
| $NO_3$ | 0.014 | 0.0134 | 0.0127 | 0.0114 | 0.0101 | 0.0089 | 0.0062 | 0.005 |
| $NH_4$ | 0.008 | 0.0077 | 0.0071 | 0.0062 | 0.0054 | 0.0046 | 0.0029 | 0.002 |
| Org. | 0.030 | 0.028 | 0.026 | 0.022 | 0.018 | 0.015 | 0.007 | 0.003 |
| Part | 8000 | 7600 | 7000 | 6000 | 5000 | 4000 | 1700 | 1000 |
| pH | 6.0 | 7.0 | 7.5 | 7.75 | 8.0 | 8.1 | 8.2 | 8.2 |

* Concentration expressed as nM

Carbonate concentration set at $2 \times 10^{-4}$M

**Table 6 Comparison of MINEQL model runs (literature data from 88)**

| Species | River Water (log Molality) | | | Seawater (log Molality) | | |
|---|---|---|---|---|---|---|
| | Lit. | This run | Ratio | Lit. | This run | Ratio |
| $Ca^{++}$ | 3.54 | 3.53 | 1.00 | 2.11 | 2.24 | 0.94 |
| $CaSO_4$ | 5.55 | 5.62 | 0.99 | 3.00 | 2.96 | 1.01 |
| $CaHCO_3$ | 5.22 | 5.72 | 0.91 | 4.84 | 5.60 | 0.86 |
| $CaCO_3$ | 5.82 | 5.72 | 1.02 | 5.30 | 5.40 | 0.98 |
| $Mg^{++}$ | 3.54 | 3.52 | 1.01 | 1.34 | 1.74 | 0.77 |
| $Na^{+}$ | 3.28 | 3.28 | 1.00 | 0.34 | 0.34 | 1.00 |
| $K^{+}$ | 4.45 | 4.46 | 1.00 | 2.01 | 1.99 | 1.01 |
| $KSO_4^{-}$ | 7.58 | 7.59 | 1.00 | 3.46 | 3.34 | 1.04 |
| $Cr^{+++}$ | 16.15 | 16.11 | 1.00 | 24.55 | 16.70 | 1.47 |
| $Fe^{++}$ | 15.18 | 6.60 | 2.30 | - | - | |
| $Fe^{+++}$ | 20.64 | 20.66 | 1.00 | 20.26 | 20.62 | 0.98 |
| $Ni^{++}$ | 7.93 | 7.51 | 1.06 | 7.92 | 7.70 | 1.03 |
| $Cu^{++}$ | 10.42 | 9.90 | 1.05 | 11.32 | 9.97 | 1.14 |
| $Cu(OH)_2$ | 13.22 | 8.14 | 1.62 | - | - | |
| $Zn^{++}$ | 8.48 | 8.45 | 1.00 | 8.06 | 7.86 | 1.03 |
| $Cd^{++}$ | 9.41 | 9.10 | 1.03 | 10.70 | 10.66 | 1.00 |
| $CdOH^{+}$ | 11.46 | 10.14 | 1.13 | - | - | |
| $CdCl^{+}$ | - | - | | 9.41 | 9.47 | 0.99 |
| $CdCl_2$ | - | - | | 9.48 | 9.32 | 1.02 |
| $Pb^{++}$ | 11.93 | 11.29 | 1.06 | - | - | |
| $Al^{+++}$ | 17.16 | 16.14 | 1.06 | 16.15 | 16.81 | 0.96 |
| $Al(OH)_3$ | - | - | | 7.58 | 8.00 | 0.95 |
| $Al(OH)_4^{-}$ | 8.37 | 6.86 | 1.22 | 7.32 | 7.16 | 1.02 |
| $PO_4^{3-}$ | 11.40 | 10.21 | 1.12 | 11.76 | 10.45 | 1.13 |
| $HPO_4^{2-}$ | 7.16 | 6.28 | 1.14 | 8.48 | 7.36 | 1.15 |
| $H_2PO_4^{-}$ | 8.06 | 7.15 | 1.13 | 10.06 | 8.86 | 1.14 |
| $HCO_3^{-}$ | 2.93 | 3.73 | 0.79 | 3.39 | 4.48 | 0.76 |
| $CO_3^{-}$ | 5.12 | 5.93 | 0.86 | 4.89 | 6.06 | 0.81 |
| $Cl^{-}$ | 3.55 | 3.55 | 1.00 | 0.25 | 0.29 | 0.86 |
| $SO_4^{-}$ | 4.14 | 4.14 | 1.00 | 1.89 | 1.92 | 0.98 |

The majority of the species considered show close agreement between the models. The main areas of disagreement were for Fe(II), the hydroxylated species of copper and aluminium, the phosphate species and the carbonate and bicarbonate ions.

The most probable explanation for the discrepancies noted above, is the way that MINEQL-N equilibrates the test solutions with super-saturated solids. This is highlighted by Nordstrom, noting that P, Ca, Fe, Al and Si species are not comparable for this reason. Comparison of the data from the two MINEQL programs with a typical program, EQUIL, (89), which does not equilibrate the super-saturated solids, supports this suggestion, see table 7.

The results obtained by MINEQL-MHIC were preferred, as this approach led to predictions of trace metal speciation which more closely resembled the dynamic situation which was to be investigated.

**Table 7 Comparison of results between EQUIL and MINEQL (values - log Molarity)**

| Species | MINEQL-N | MINEQL-MHIC | EQUIL |
|---|---|---|---|
| $Fe^{++}$ | 15.18 | 6.60 | 6.71 |
| $Cu(OH)_2$ | 13.22 | 8.14 | - |
| $Al(OH)_4^-$ | 8.87 | 6.86 | 7.09 |
| $PO_4^{3-}$ | 11.40 | 10.21 | 10.03 |
| $HPO_4^{2-}$ | 7.16 | 6.28 | 5.82 |
| $H_2PO_4^-$ | 8.06 | 7.15 | 6.69 |

### 2.3.2 Comparison of data from seawater model

The data obtained from the seawater run were more problematical, as there were initially several differences between the two runs not obviously associated with the difference in approaches, noted in section 2.3.1.

The ions, Ca and associated carbonate species, and the phosphate ions may be assumed to be affected in a similar way in seawater as described above for freshwater. However, the correlation with other programs, *e.g.* EQUIL, was not as satisfactory. The most probable explanation relates to the uncertainties that exist about the form of dissolved carbon dioxide, and its concentration. Differing modelling programs handle this variable in different ways, thus MINEQL will allow a concentration to be part of the input data, but may also vary this value, depending on whether the gas is assumed to be in equilibrium with the test solution. For the purposes of this exercise, these differences were acceptable.

More worrying were the differences between the free metal ions, *e.g.* $Cu^{++}$ and $Cr^{+++}$. However, these metals are dominated by complexation with the hydroxide ion, and a small change in either the formation constant of these species, or in the calculated hydroxyl concentration will lead to large changes in the free ion concentration.

In conclusion, although for a number of ions there were

differences between the two MINEQL programs, the most probable explanation relates to the different approaches to supersaturated solids. The results obtained were not, though, inconsistent with other equilibrium programs.

## 2.4 CHROMIUM SPECIATION IN MODEL ESTUARIES

These initial experiments were designed to investigate the potential changes in chromium speciation during its passage from a riverine input through to the sea. The data used were as described above in table 5, and these data were amended during the runs in order to investigate the effect of such changes on chromium.

### 2.4.1 Basic model

The basic model, without organics or particulates, predicted that chromium would initially be in the forms $Cr(OH)^{++}$ and $Cr(OH)_2^{+}$, with $Cr(OH)_4^{-}$ being the dominant form as salinity increased, figure 3a. These changes are in effect a reflection of the changes due to pH rather than salinity, and a scan of the freshwater, with changing pH showed this to be the case, figure 3b.

### 2.4.2 Effect of redox on the basic model

The effect of introducing redox reactions into the model was not unexpected, and resulted in complete oxidation of chromium(III) to (VI). This occurs because the model is

an equilibrium model, and the transfer of chromium(III) to chromium(VI) is slow, see section 1.3, such that in the environment chromium(III) will still be determined. Furthermore, the adsorption of chromium to particulates, or complexation to hydroxide forms will inevitably introduce further kinetic control.

For this reason the remaining model runs were all carried out in the absence of redox reactions.

### 2.4.3 Effect of particulates on the basic model

Using the data described above, there was an initial effect on the speciation of chromium(III) due to the presence of particulates, figure 3c. However, this rapidly disappeared with increasing salinity. That this was probably due to the response altering with changes in pH was confirmed by investigating the effect of changing the pH on chromium(III) adsorption, figure 3d. Although the extent of the predicted chromium adsorption does not reach the levels reported in environmental monitoring exercises, up to 40%, there was some internal consistency of the data, in that the impact on copper, lead and zinc was also lower than that found in monitoring studies, for example in the Tees, (93), by Comber and Eales. Thus copper and lead were found to be bound at levels up to 90% in the reported study, while the model predicted 12% adsorption of lead, and 8% of copper, in freshwater.

**Figure 3 Speciation of chromium in an estuarine model**

Figure 3a : Basic Model

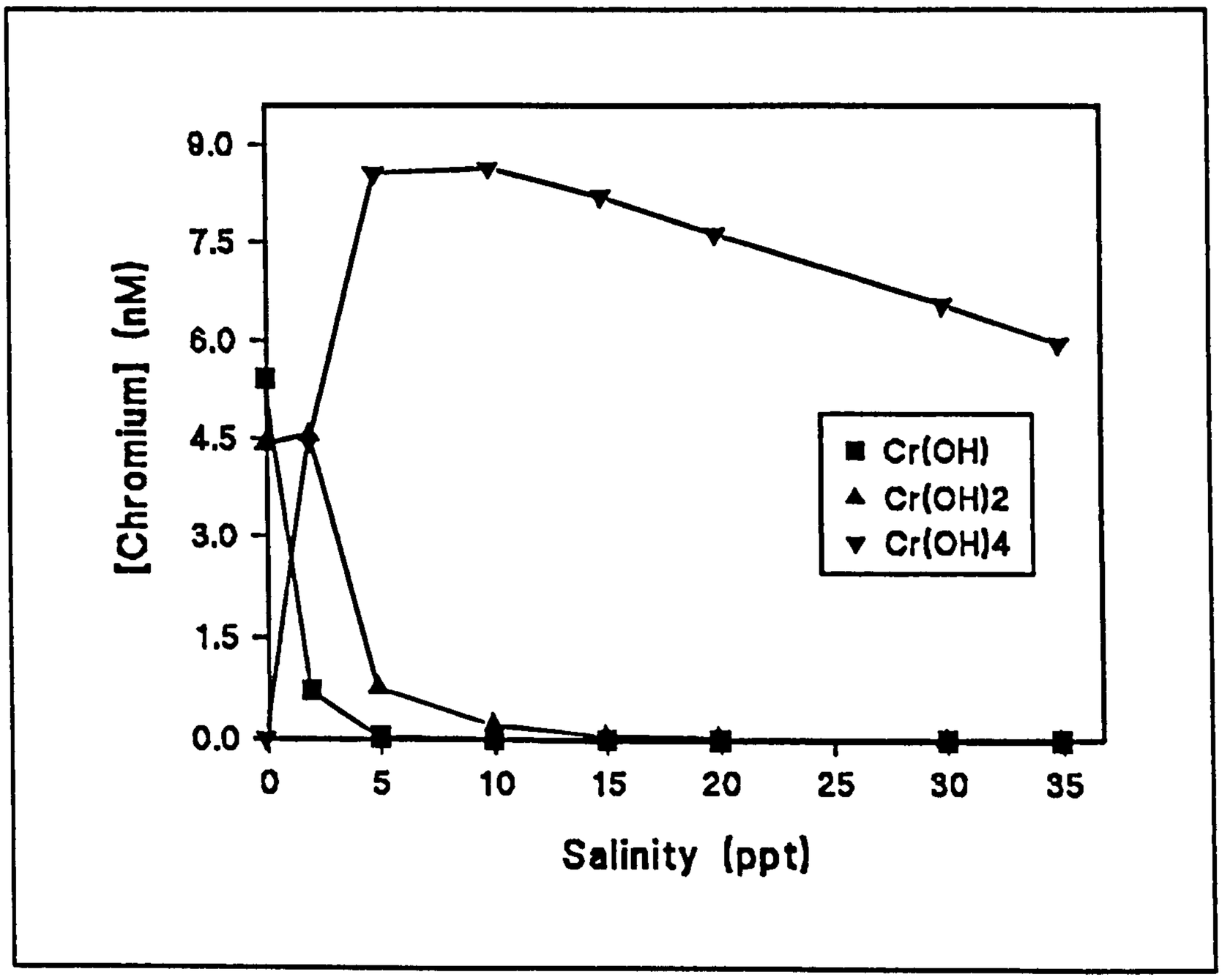



Figure 3b : Effect of pH on Cr(III) speciation

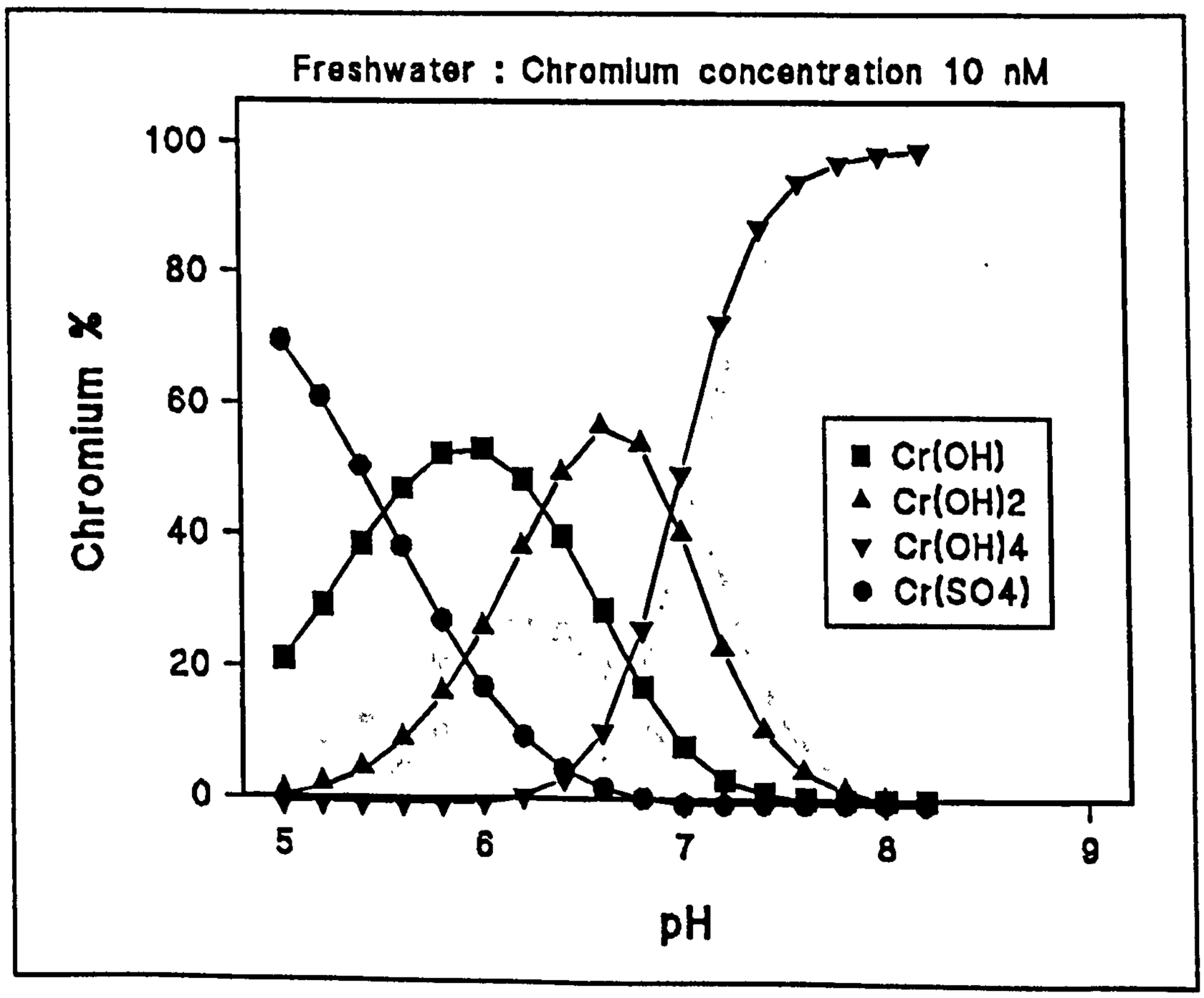


**Figure 3 Speciation of chromium in an estuarine model (cont)**

Figure 3c : Basic Model + Particulates

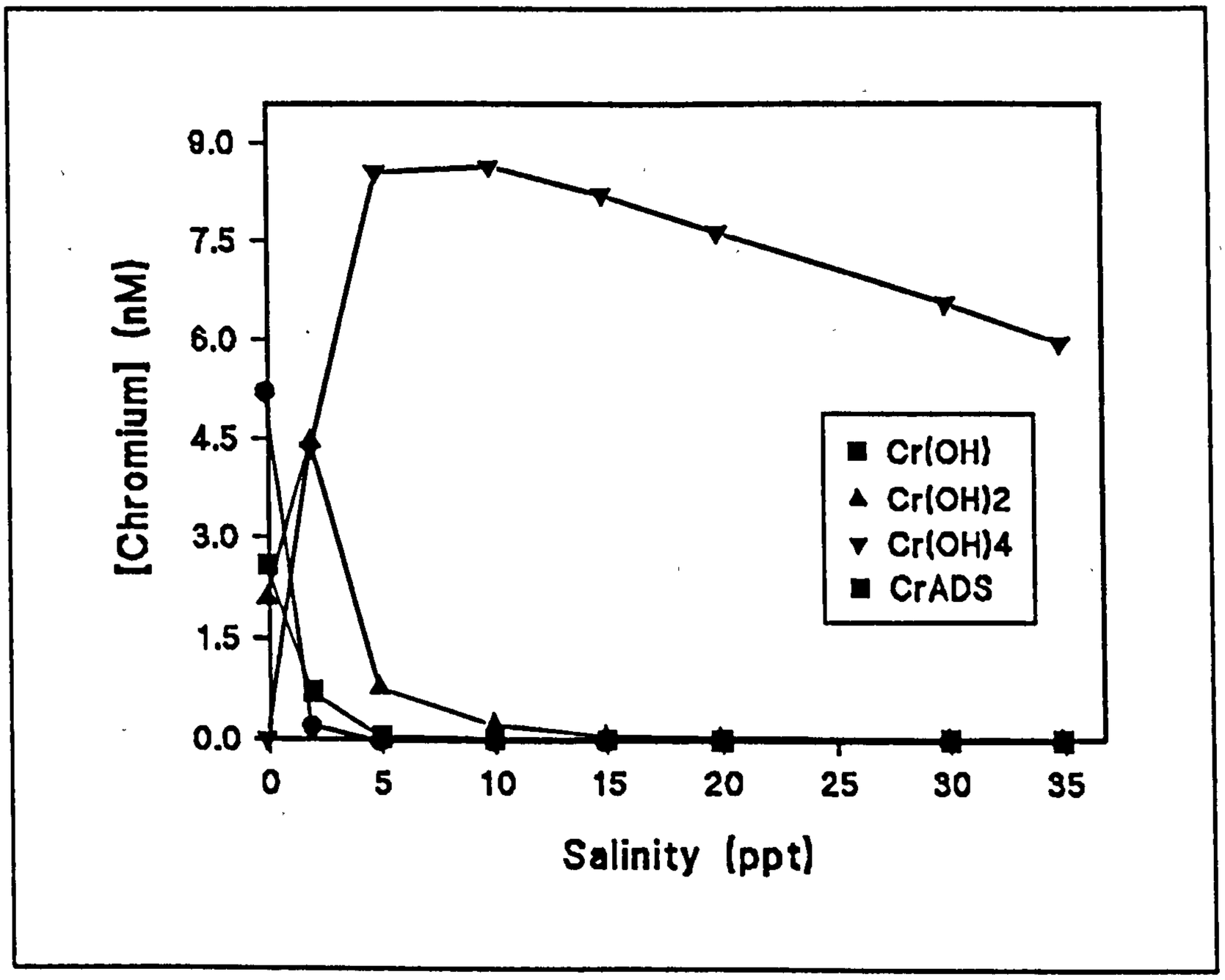
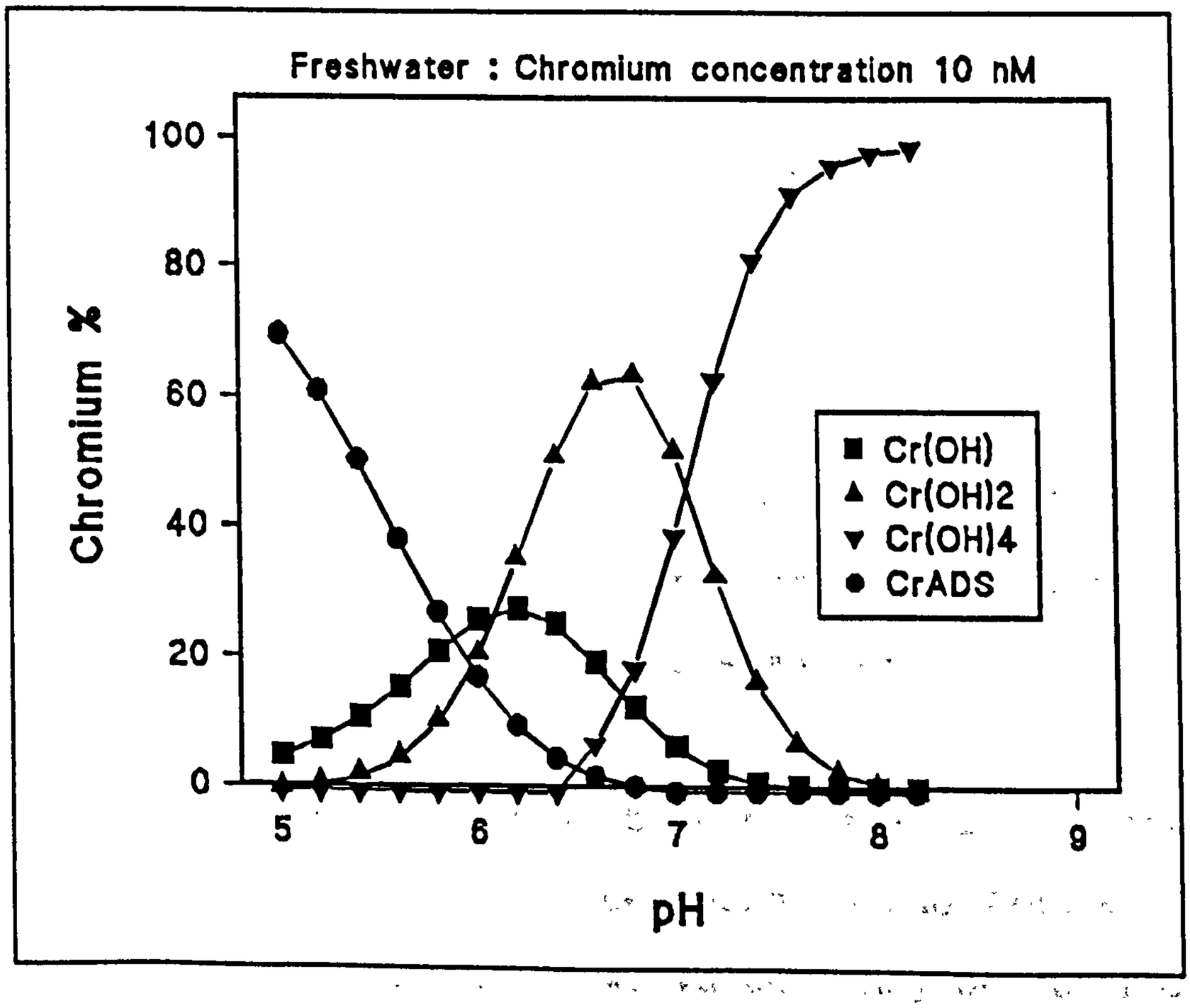



Figure 3d : Effect of pH on Cr(III) adsorption



There are two possible explanations for this discrepancy, either the stability constants are an underestimate for the particulate matter in the Tees, or the concentrations in the Tees are much higher. It was proposed to investigate further the impact of these two variables when the survey of the Tees had been completed, and further information regarding the proportion of bound metal and concentration of particulate matter was known.

### 2.4.4 Effect of organic interactions on the basic model

As described in section 2.2.4 two organic chelators were used to investigate the potential impact of organic matter on chromium. The first one reported here was citric acid, which as a carboxylic acid, is not dissimilar to a large proportion of the organic matter considered to be present in estuaries. However, while the pattern obtained for copper was not dissimilar to that found by other workers, *i.e.* falling from a level in excess of 90% in freshwater, to approximately 50% in seawater, there was little impact on chromium(III).

This was in total contrast to the effect of EDTA which completely complexed both chromium and copper at all salinities up to that of full seawater.

The suggestion from this, that the stability constants for the complexing matter to be found in the Tees would lie somewhere between these two values, may be further

investigated again following the survey of the Tees. Thus, once a value for the amount of chromium(III) complexed by organic matter and the concentration of that organic matter had been obtained, it should then be possible to obtain an approximate average stability constant using MINEQL.

### 2.4.5 Full model

In view of the uncertainty of the actual levels of both particulate and organic matter, and their associated stability constants, it was decide that the interactions of these two could be left until more information had been obtained.

Following from the Tees monitoring exercise, it would then be necessary to estimate using MINEQL, the stability constants for both sets of complexing agents.

## 2.5 MODEL OF ELECTROCHEMICAL CELL MEDIA

To enable a better understanding of the response of chromium in the electrochemical cells, examined by cyclic voltammetric scans, it was decided to model the behaviour of various constituents. Initially the effect of salinity and pH on diethylene-triamine-pentaacetic acid, DTPA was examined. This was necessary as it would help in selection of the optimum pH for the electrochemical determination of chromium. It was also seen as a

necessary step, to aid in the understanding of the response of chromium in an estuarine environment, where the increasing ionic content of the waters was expected to lead to competition for DTPA.

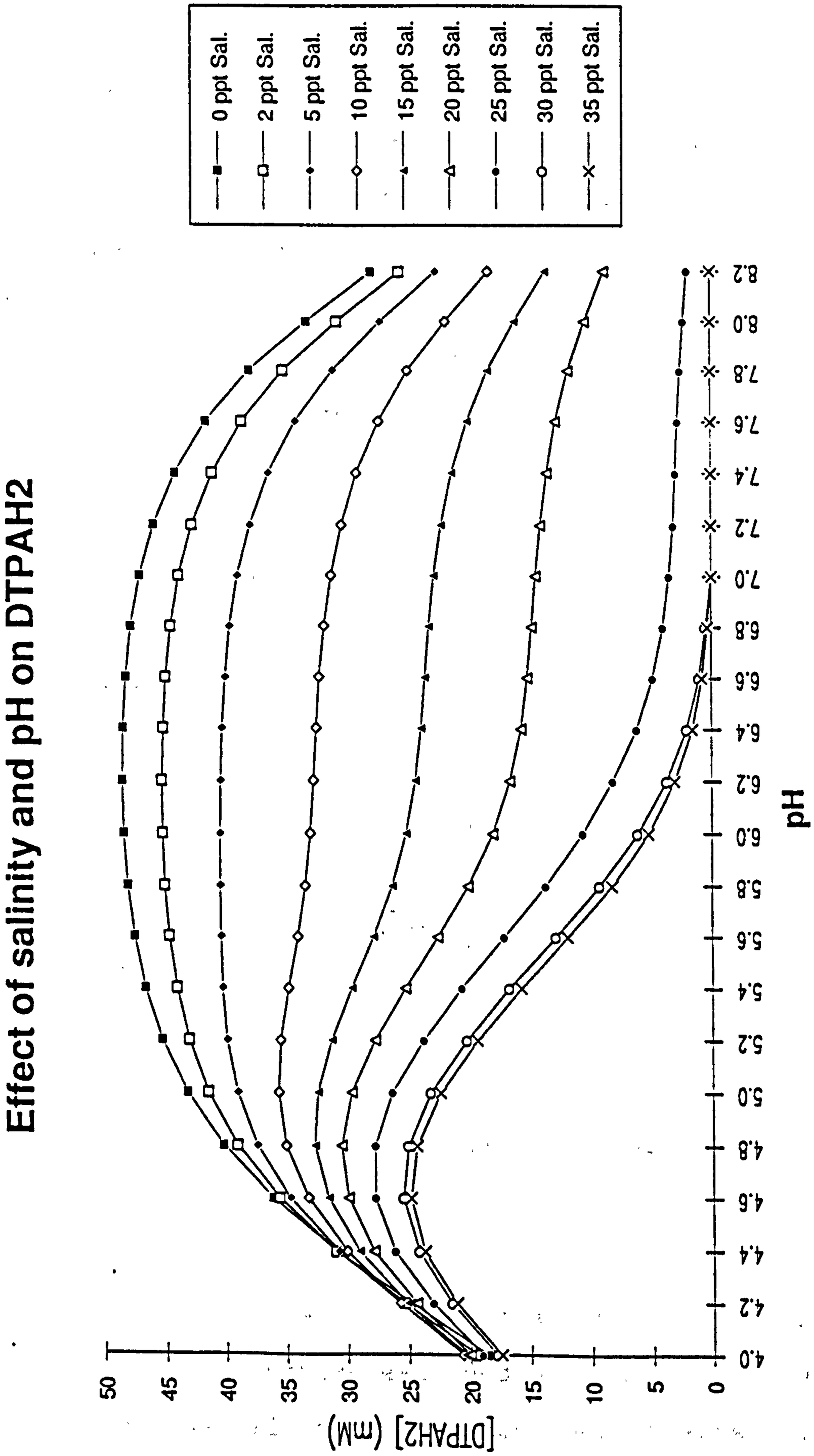
Later experiments, as will be described, were carried out with constituents of Britton & Robinson buffer, *i.e.* boric acid, phosphate, barbitone and citric acid. The speciation of these constituents was also determined.

### 2.5.1 Effect of pH and salinity on diethylene-triamine-pentaacetic acid, (DTPA)

The model data for this experiment is shown in figure 4, which describes the effect of pH and salinity on the availability of $DTPAH_2$, which is considered to be the most likely form interacting with chromium(III) in the electrochemical cell, (25,71).

This distribution shows that as salinity increases, the pH for the maximum concentration of $DTPAH_2$ decreases. The optimum pH for the chromium(III) response in seawater from this study is approximately 4.6. This differs from that described by Boussemart *et al.*, (25), in that the concentration of $DTPAH_2$ was estimated in seawater, with magnesium present at 53 mM, and the optimum pH was 5.05. However, the major difference was that Boussemart *et al.* also predicted that the concentration of $DTPAH_2$ would only be approximately 1 μM. In an attempt to understand this

**Figure 4** **Effect of pH and salinity on the distribution of DTPA forms**



difference, MINEQL was used to predict the speciation of DTPA in the presence of magnesium alone, and then with chloride added. The concentrations of magnesium and chloride were those described in table 5, for seawater. This showed that if only magnesium was present with DTPA, the level of $DTPAH_2$ was less than 20 μM, not too dissimilar to that suggested by Boussemart. However, as soon as seawater constituents were added, in this case chloride, the level of $DTPAH_2$ increased to over 100 μM. The reason for this is due to the complexation of magnesium by chloride, and thus a reduction in the concentration of free magnesium available to react with DTPA. It is probable that further constituents will similarly reduce the level of available magnesium, leading to an increase in the concentration of $DTPAH_2$. While there will be differences between the actual values obtained, due to differing stability constants of the various components, the broad picture will remain essentially the same. Thus in seawater there will be a reduction in sensitivity over that obtained in freshwater, and the optimum pH for determination of chromium will be approximately pH 5.

### 2.5.2 Modelled behaviour of Britton & Robinson buffer constituents

The basic model was based on the acetate buffer, described below. Each of the systems examined were considered at pH 6.2 and 8, and the Britton & Robinson constituents were added at 0.02 M. The effect of the presence of

chromium(III) was also considered, as the changes in complexation would help understand the behaviour of chromium in the cyclic voltammetry experiments.

### 2.5.2.1 Acetate buffer

The forms of acetic acid and DTPA present at pH 6.2 and 8 are shown in table 8. There was little difference between the major forms found at these pH, but there was a clear difference in the speciation of chromium(III). This is not considered to alter the electrochemical response.

The chromium speciation remained consistent with that first obtained in acetate buffer, and did not alter with the addition of constituents of Britton & Robinson buffer. As a result the data relating to chromium has only been recorded for the acetate buffer.

### 2.5.2.2 Acetate buffer with boric acid

The $pK_a$ of boric acid, $B(OH)_3$, is 9.2. Calculating the % distribution of the two forms, from the equation;

$$B(OH)_3 + OH^- \text{ ==== } B(OH)_4^-$$

and using K = 9.2, gives 0.1% of $B(OH)_4^-$ at pH 6.2, and 6% at pH 8. The effect of these changes on the electrochemical response of chromium(III), will be discussed in section 3.

### 2.5.2.3 Acetate buffer with citric acid

The distribution of citric acid forms at pH 6.2 and 8 is shown in table 8. The effect of these changes is more likely to impact on the reduction of chromium(VI) than any complexation of chromium(III). This arises out of the modelling exercise discussed in section 2.4.4 above.

### 2.5.2.4 Acetate buffer with phosphate

The effect of pH, at 6.2 and 8, on the speciation of phosphate is shown in table 7.

**Table 8 Speciation models in cyclic voltammetry cells, with added phosphate or citric acid**

| Buffer | Component | % @ pH = 6.2 | % @ pH = 8.0 |
|---|---|---|---|
| Acetate | $DTPAH_1$ | - | 8.7 |
| | $DTPAH_2$ | 97.1 | 91.3 |
| | $DTPAH_3$ | 2.7 | - |
| | $Acetate^-$ | 91.3 | 100 |
| | H Acetate | 8.7 | - |
| | Cr(OH) | 56.8 | <0.1 |
| | $Cr(OH)_2^+$ | 42.0 | 1.2 |
| | $Cr(OH)_4^-$ | <0.1 | 98.8 |
| Acetate + phosphate | $H_4(PO_4)_2^-$ | 1.2 | <0.1 |
| | $H_2PO_4^-$ | 75.8 | 4.8 |
| | $HPO_4^-$ | 15.0 | 69.2 |
| | $KHPO_4^-$ | 8.0 | 25.9 |
| Acetate + Citric Acid | $CTA^-$ | 79.5 | 99.6 |
| | $CTAH^-$ | 20.2 | 0.4 |

### 2.5.2.5 Acetate buffer with barbitone

Based on its $pK_a$ of 7.98, at pH 6.2, 1.6% of the barbitone will be unprotonated, and at pH 8, 51% will be unprotonated. There is little known about the effect of barbitone on DTPA or chromium, and whether this change leads to an altered chromium(III) electrochemical response will be further discussed in the next section.

# CHAPTER 3.....ELECTROCHEMICAL INVESTIGATIONS

## 3.1 INTRODUCTION

As described in section 1.5.6, the adsorption system used was originally described by Golimowski, *et al.*, (71), for the determination of total chromium. The principal steps in their method are outlined below.

i. Pretreatment of the sample to chemically oxidize all the chromium to chromium(VI).

ii. Chromium(VI) was then reduced in the electro-chemical cell at the plating potential of -0.9V. The chromium(III) formed immediately complexed with DTPA already adsorbed on the mercury drop.

iii. This complex was then stripped from the drop by differential pulse polarography, scanning from -0.9V to -1.4V.

iv. At -1.27V the chromium(III) was reduced to chromium(II), and a peak proportional to the chromium concentration in the solution produced.

Initially it was decided to investigate this method using cyclic voltammetry, (CV). The use of CV enables chemical and electro-chemical reactions to be studied in the same

system. A further advantage of this approach is the ability to investigate kinetic effects, by altering the electro-chemical scanning rate.

In the initial experiments, three electrochemical responses were examined.

| | | | |
|---|---|---|---|
| Cr(III) | -> Cr(II) | - cathodic | (reaction a) |
| Cr(II) | -> Cr(0) | - cathodic | (reaction b) |
| Cr(II) | -> Cr(III) | - anodic | (reaction c) |

It was also intended to investigate alternative complexing reagents for their potential to selectively complex and hence determine the two oxidation states of chromium.

## 3.2 EQUIPMENT AND CHEMICALS

A PAR 384B Polarographic Analyzer with a 303 Hanging Mercury Drop Electrode, HMDE, obtained from EG & G, Bracknel, Berkshire was used. The working electrode had a surface area of 1.7 $mm^2$. A platinum counter electrode, and a silver/silver chloride reference electrode, (Ag/AgCl) were used. All the potentials were measured with respect to the Ag/AgCl electrode. The current-potential curves were stored by the 384B, then scaled and recorded on a Houston Instrument Model DMP-40 plotter, obtained from EG & G.

The CV scans were carried out at room temperature, in PTFE

cells, with 10 ml of solution present. The solutions were purged with purified nitrogen, (minimum 99.999% pure), prior to experimentation. Cathodic stripping voltammetry, CSV, experiments were carried out in similar cells, any operational differences will be described later.

The basic operating parameters used were :

| | |
|---|---|
| Initial Potential | -0.9 V |
| Final Potential | -1.7 V |
| Plating time | 30 s at -0.9 V |
| Equilibration time | 10 s |
| Scan Rates | 10-1000 mV s-1 |

All chemicals used in this work were obtained from BDH Chemicals, Poole, Dorset, except for those used in the preparation of the Britton & Robinson buffer, which were obtained from the Sigma Chemical Co. Poole, Dorset.

Buffer control at pH 6.2 was achieved by either adding 100 µl of 1 M sodium acetate to Milli-Q water, or using Britton & Robinson buffer.

Britton & Robinson buffer, (94), was made up of 0.022 M citric acid, 0.02 M diethylbarbituric acid, 0.02 M boric acid, 0.02 M potassium dihydrogen phosphate and 0.06 M sodium hydroxide. The buffer was prepared by taking the appropriate weights and adding them directly to water. After stirring, normally overnight, this solution was made

up to the appropriate volume, normally a litre. When tested, the pH of was found to be 6.2 +/- 0.1. This buffer was then used directly as the blank solution, prior to addition of chromium.

$1x10^{-4}$ M DTPA was added to all solutions, prior to running a blank CV scan, after which an appropriate concentration of chromium(III), as $CrCl_3$, and chromium(VI), as $K_2CrO_4$, was added.

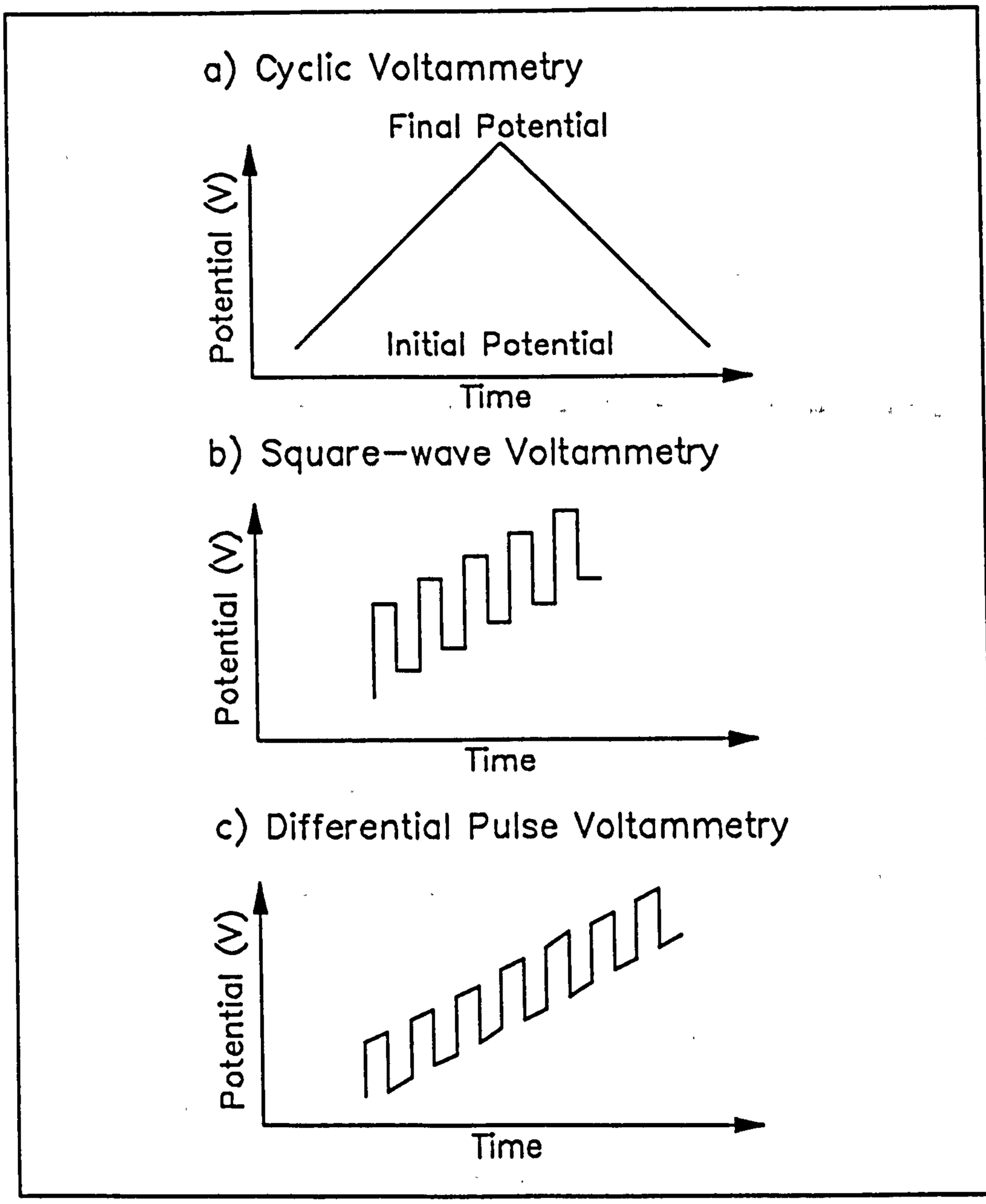
## 3.3 CYCLIC VOLTAMMETRY INVESTIGATIONS

In Cyclic Voltammetry, (CV), the current in the cell is measured while a triangular potential sweep occurs, figure 5a. This results in cathodic and anodic current responses, and with the rapid scans available, means that redox responses and their kinetic aspects may be investigated.

### 3.3.1 Reproducibility of chromium(III) response

An initial study was undertaken to explore the reproducibility of the system under investigation. It was also to investigate if there was a difference between the reactivity of chromium present in solution *i.e.*from a previously prepared solution of chromium, and that added to the cell, *e.g.* as a 100 µl spike from a much stronger stock solution. This was felt necessary, given the generally slow kinetics of chromium(III) reactions. This

**Figure 5 Voltammetric potential waveforms**




would also check the feasibility of spiking a solution in the cell, were it to be a necessary step for quantification.

The results obtained, table 9, demonstrate there was no difference between the two approaches, and that the reproducibility was generally acceptable at around 5% RSD,

except for the chromium(II) reduction peak. The effect of the two treatments on the chromium(II) reduction peak was significant, (*p=0.05, Students T test*), although the variability of this response was higher than for the other responses. The reasons for this significance are not clear, but it was not investigated any further at this stage as this response was unlikely to be useful.

**Table 9 Mean peak responses from CV experiments - chromium(III), (n = 12)**

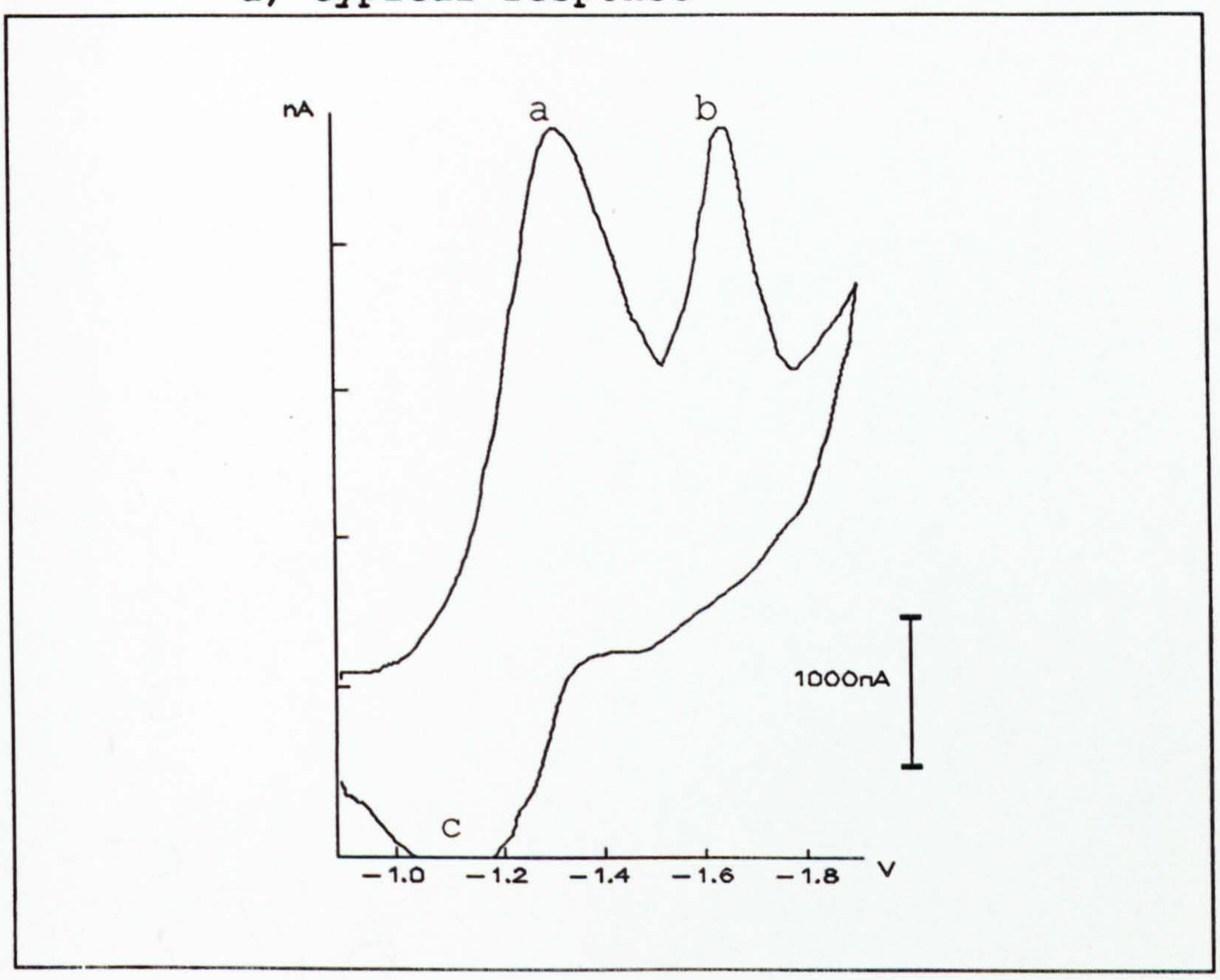
| | Cr(III) -> Cr(II) | | Cr(II) -> Cr(0) | | Cr(II) -> Cr(III) | |
|---|---|---|---|---|---|---|
| | Peak current (nA) | Peak potential (mV) | Peak current (nA) | Peak potential (mV) | Peak current (nA) | Peak potential (mV) |
| Bulk | 1210 | -1.35 | 1036 | -1.63 | 615 | -1.27 |
| %RSD | 4.95 | | 12.2 | | 6.3 | |
| Spike | 1201 | -1.35 | 1150 | -1.63 | 590 | -1.27 |
| %RSD | 5.8 | | 7.2 | | 4.4 | |

It was also apparent from this experiment that the response at the electrode for Cr(II) -> Cr(0) was considerably less than that of the chromium(III) reduction, even though the former was a two electron process.

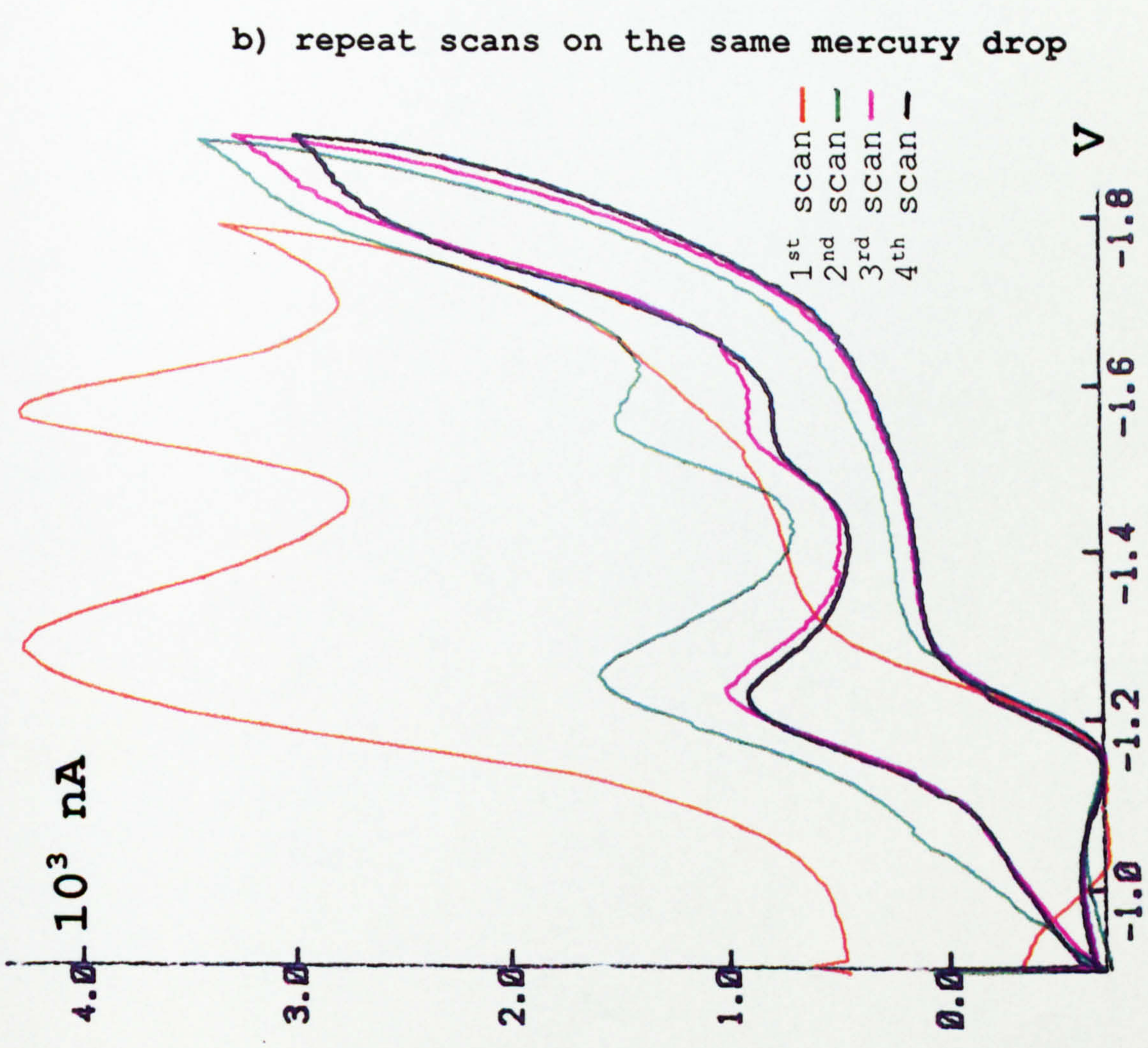
This is probably due to the slow kinetics of this reduction process, and this will be discussed later, section 3.3.2.

Figure 6a shows a typical scan, obtained during these experiments, with a scan rate of 400 mV $s^{-1}$.

**Figure 6** Cyclic Voltammetric Scans of chromium(III)
a) typical response




b) repeat scans on the same mercury drop



The scan shows three peaks, which may be assigned to;

Cr(III)-DTPA -> Cr(II)-DTPA cathodic @ -1.35V (a)

Cr(II)-DTPA -> Cr(0) cathodic @ -1.63V (b)

Cr(II)-DTPA -> Cr(III)-DTPA anodic @ -1.17V (c)

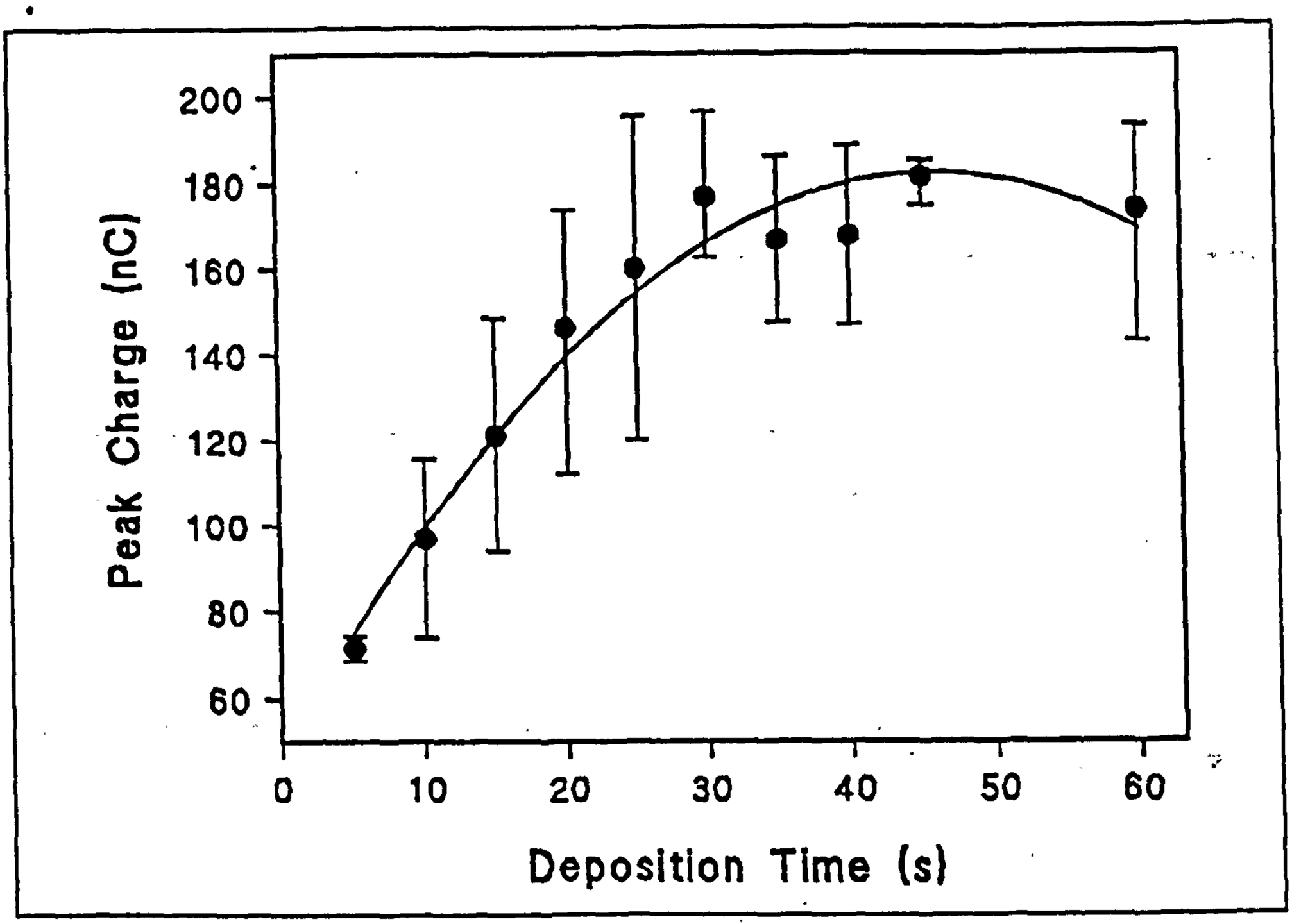
where a, b and c refer to the labels in figure 6a.

Figure 6b in which a number of scans were performed on the same drop, shows a number of features. The response due to reaction a, chromium(III)-DTPA reduction, declines with the number of scans, and shifts in a positive direction. The reduced response is probably due to chromium(III)-DTPA diffusing from the surface of the electrode. While the shift in the initial reduction peak is probably due to the formation of secondary layers during the deposition period. This was investigated by changing the deposition time, figure 7, which shows that at the deposition time of 30 seconds the response is at a maximum, suggesting that the drop was covered in full by the chromium-DTPA complex.

The absence of an oxidation peak for Cr(0) confirms that this is irreversible, (62). This response also declines as the number of scans increases, probably because there is less Cr(II)-DTPA being formed.

It is concluded from these experiments that reaction a is reversible, but reaction b is not. The latter is also a much reduced response, probably due to slow kinetics. This was investigated by varying the scan rate at which the cyclic voltammetry was conducted, section 3.3.2.

Figure 7 Chromium(III) response versus Deposition time




### 3.3.2 Effect of Cyclic Voltammetric scan rate

Using the same conditions as previously described, the scan rate was varied from 40 to 1000 $mVs^{-1}$. The results are shown in table 10 for the redox pair, Cr(III) -> Cr(II), and table 11 for Cr(II) -> Cr(0).

Initial examination of the data suggested that the chromium(III)-DTPA response was reversible, because the peak current increased with the square-root of the CV rate. However, it was not proportional, *i.e.* I/Rate was not constant, and so the reaction should be classed as irreversible in this system. Similarly, the ratio of the

**Table 10 Cyclic voltammetric characteristics of chromium(III) - chromium(II) responses in the presence of DTPA**

Cr(III)-DTPA --> Cr(II)-DTPA --> Cr(III)-DTPA

| Rate ($mVs^{-1}$) | $I_p^C$ (nA) | $\frac{I_p^C}{Rate^{0.5}}$ | $E_p^C$ (V) | $I_p^A/I_p^C$ | $E_p^C-E_p^A$ |
|---|---|---|---|---|---|
| 40 | 119 | 19 | -1.35 | 0.84 | 84 |
| 100 | 297 | 30 | -1.34 | 1.35 | 80 |
| 400 | 980 | 49 | -1.35 | 2.20 | 96 |
| 1000 | 2056 | 65 | -1.35 | 2.41 | 178 |

$I_p^C$ & $I_p^A$ : Peak current of Cathodic and Anodic peak

$E_p^C$ & $E_p^A$ : Peak potential of Cathodic and Anodic peak

**Table 11 Cyclic voltammetric characteristics of chromium(II) reduction in the presence of DTPA**

Cr(II) --> Cr(0)

| I (nA) | Rate ($mVs^{-1}$) | $\frac{I}{Rate^{0.5}}$ | E (V) |
|---|---|---|---|
| 59 | 40 | 9 | -1.56 |
| 185 | 100 | 18.5 | -1.57 |
| 829 | 400 | 41 | -1.60 |
| 1513 | 1000 | 48 | -1.61 |

reduction peak current to the oxidation peak current increased with scan rate, and the peaks moved apart also with increasing scan rate.

The chromium(II) -> chromium(0) peak demonstrated complete irreversibility, there being no oxidation peak, and a non-linear relationship between the peak potential and the square-root of the scan rate. From Nicholson & Shain,

(95), the relationship;

$$K = E_e^{\theta} - \frac{RT}{\alpha_c n_\alpha F}\left(0.78 - \frac{2.3}{2}\log\left(\frac{\alpha_c n_\alpha F D}{k^{\theta 2} RT}\right)\right)$$

predicts that at 25°C the peak shifts by $-30/\alpha_c n_\alpha$ mV every decade increase in the scan rate for an irreversible reaction. For a two electron process, n=2, and if $\alpha_c$ is taken as 0.5, the peak potential should drop by 30mV for each decade increase in the scan rate. This is in good agreement with the data in table 11.

### 3.3.3 Stability of chromium(III) response

The DTPA response relies upon the immediate formation of the chromium(III)-DTPA complex, which was described by Golimowski, (76), as occurring on the mercury drop. This hypothesis was supported by the observation that when chromium(III) was allowed to age with DTPA, and form the complex in solution, the CV response was considerably reduced, compared to that of a freshly prepared solution, figure 8.

This may also explain why the chromium(III) response decreased with time in the electro-chemical cell, (78). Initially chromium(III) present in the cell is complexed by DTPA on the mercury drop, which thus takes part in the electro-chemical stripping step. However, the DTPA also

Figure 8 Effect of time on chromium(III)-DTPA response

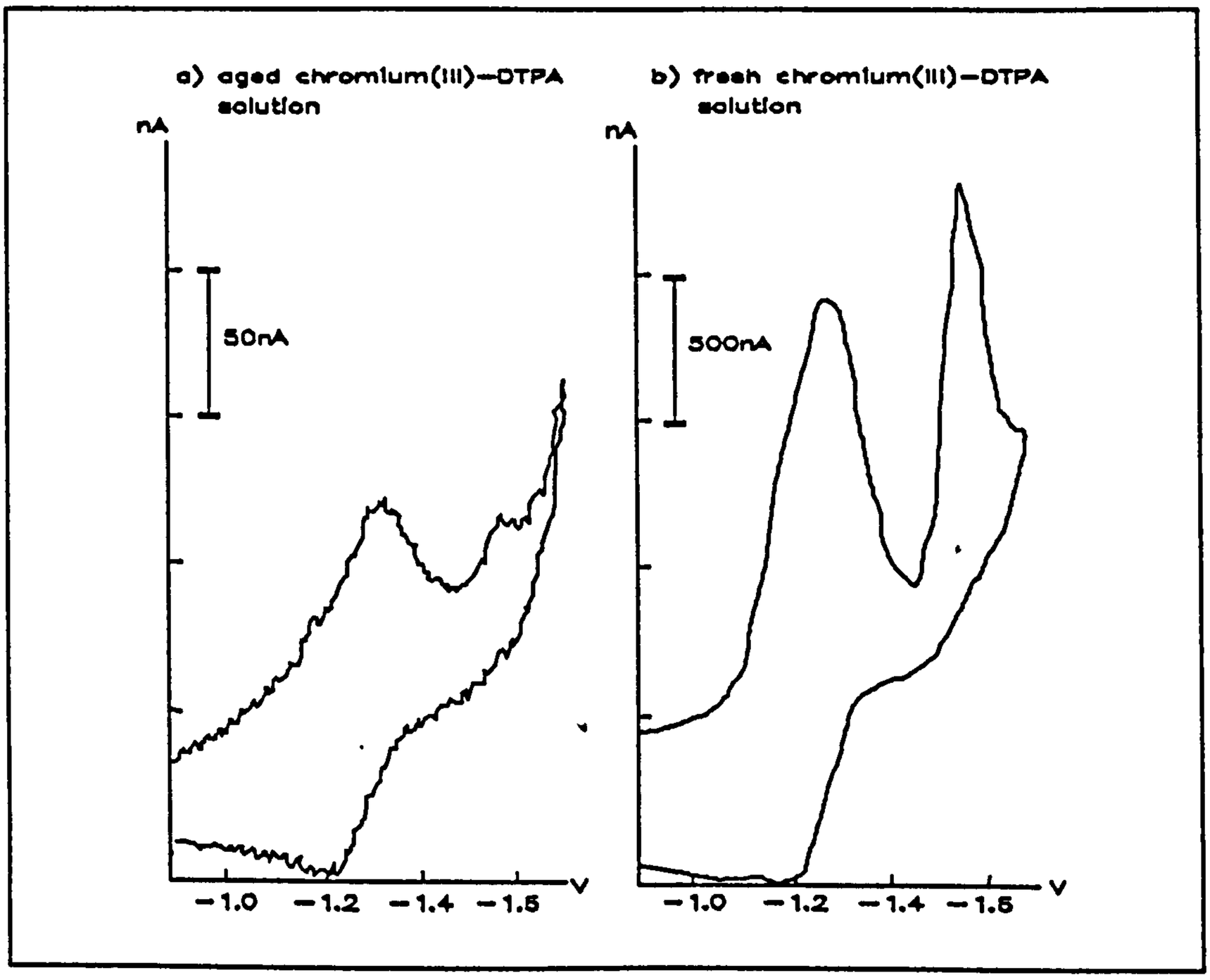
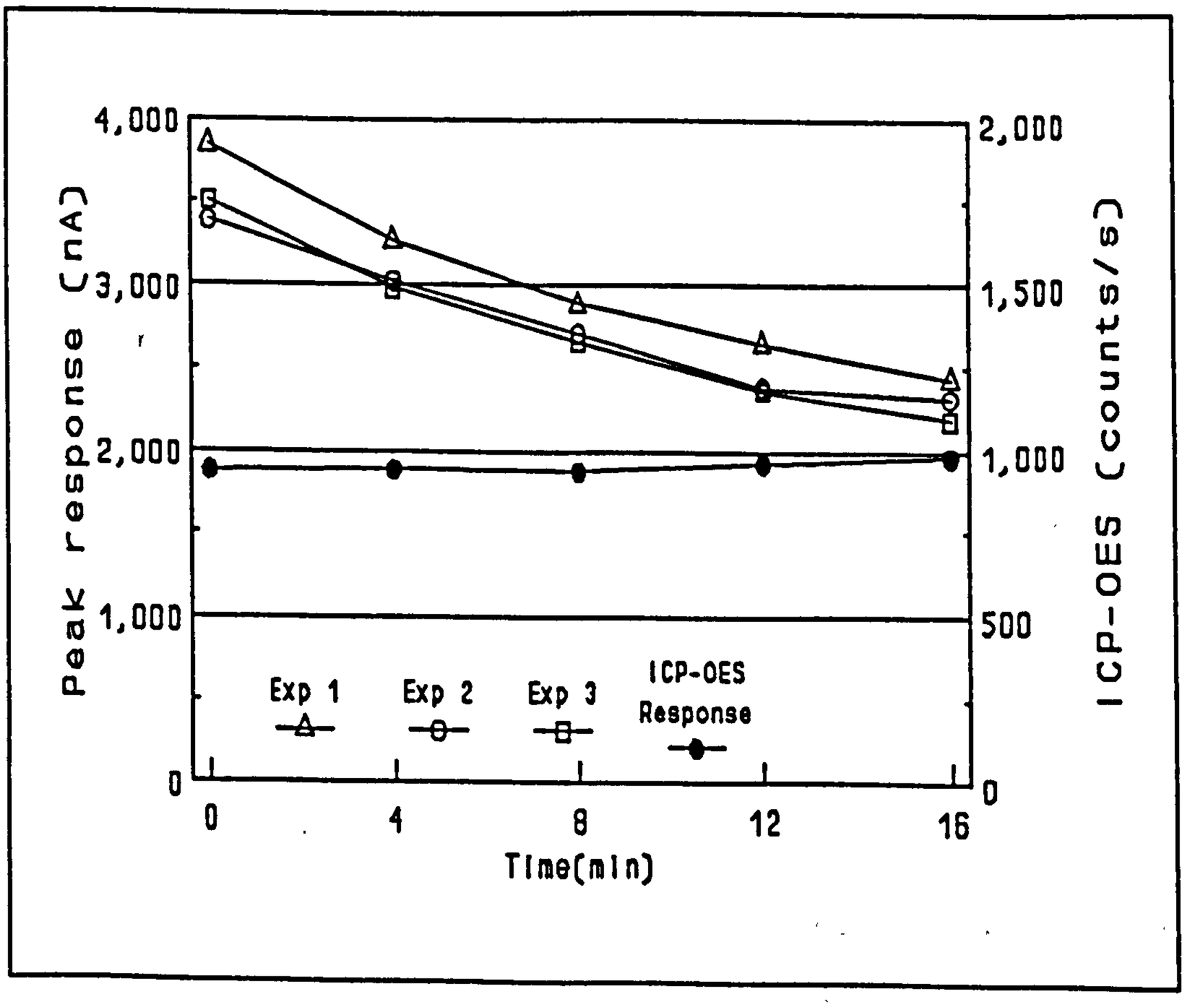



Figure 9 Stability of chromium(III) response in the electrochemical cell versus dissolved chromium



starts complexing chromium(III) in the bulk solution, and the experiment above clearly shows that this complex is inactive in the electrochemical cell, thus there is reduced available chromium(III) to be complexed at the mercury drop.

It was demonstrated that the chromium was still in solution, by analyzing parallel cups by Inductively Coupled Plasma - Optical Emission Spectrometry, ICP-OES. The results, figure 9, clearly show that although the electrochemical response dropped by 20% over 15 minutes, the chromium response by ICP-OES was constant, indicating that the chromium was still present in solution.

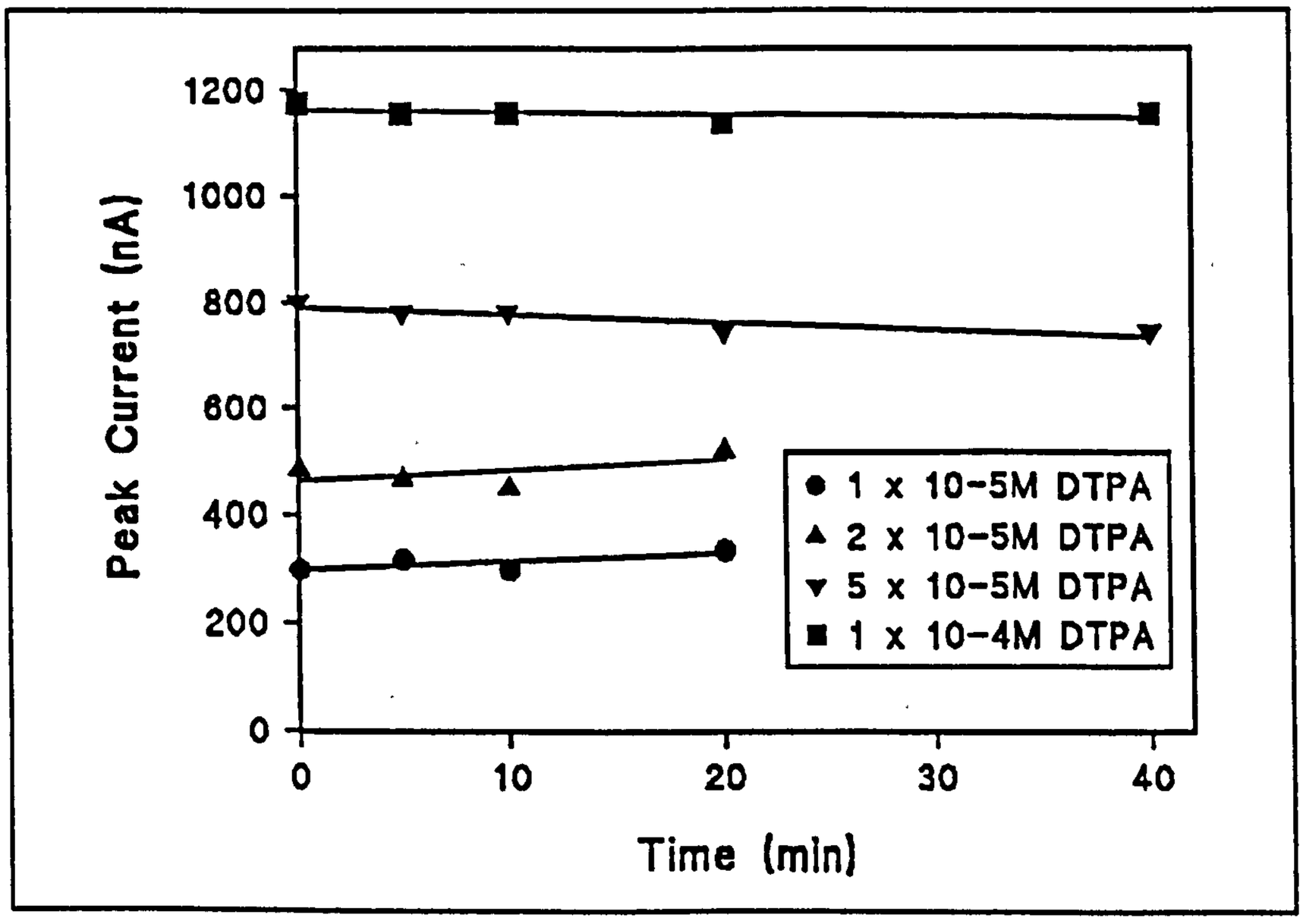
An initial examination of this declining response by CSV, was carried out, figure 10, and although at lower concentrations there was a reduction, in this experiment it did not appear to be related to the DTPA concentration. This is an area that should be further investigated, however, as when the concentration of chromium is high, *e.g.* $1x10^{-4}$ M, this effect is not apparent.

### 3.3.4 Comparison of chromium (III) and (VI) responses

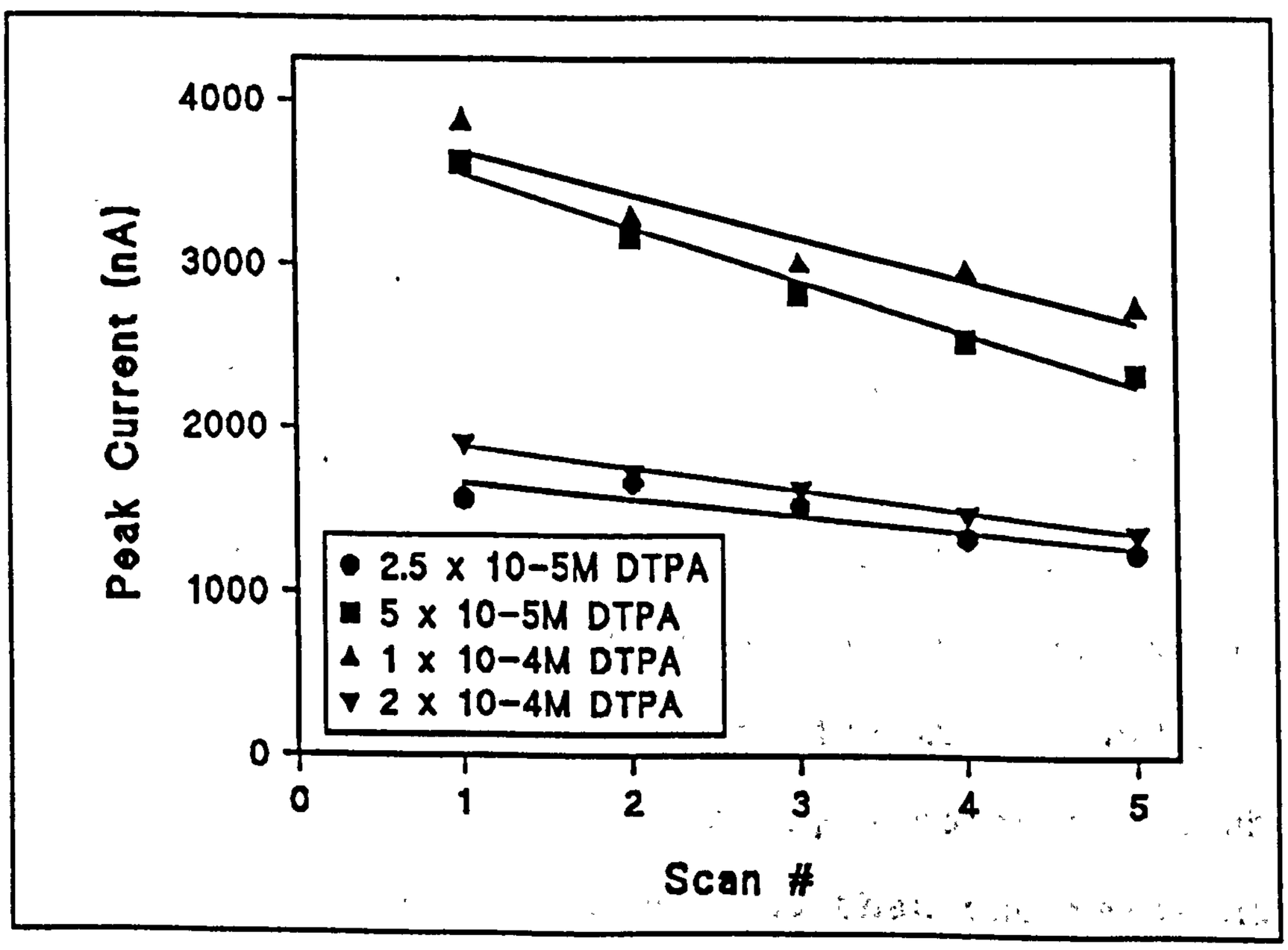
As described above the response of chromium(III), demonstrated some of the characteristics of a reversible peak, and three responses were present. However, when chromium(VI) was examined in the cell, a different picture was obtained. At 400 mV $s^{-1}$ although the proportions were

**Figure 10 Effect of DTPA concentration on chromium(III) response**

(a) Cyclic voltammetry
Cr(III) concentration 1 x$10^{-4}$M




(b) Square-wave differential pulse polarography
Cr(III) concentration 1 x$10^{-5}$M



altered, the same responses were obtained as described above, section 3.3.1. However, at lower scan rates the chromium(II) reduction peak, peak b, was lost, while at higher rates it was reduced, but still present, figure 11.

The changes in the three responses, for chromium(III) and (VI) against the CV rates are shown in figure 12.

The possible reasons for this behaviour are further discussed in section 3.3.5.

### 3.3.5 Effect of nitrate on chromium responses

Nitrate ions are added to the system to enhance the chromium(III) reduction wave by immediate re-oxidation of the chromium(II)-DTPA being formed in the cell during the stripping stage of the analysis. The evidence for this includes the enhancement, as described, and the loss of a reversible Cr(II) -> Cr(III) peak, in CV experiments, figure 13a.

The presence of chromium(VI) in the cell had the same effect as may be seen in table 12, at low CV rates, 100 mV s-1 and below. However, as the rate of the scan was increased so the effect of the chemical oxidation was lessened, until at CV rates of 400 mV s-1 and above the effect was negligible. This may also be noted when nitrate was present, suggesting that the re-oxidation step was being kinetically controlled at this point.

Figure 11 Effect of scan rate on the cyclic voltammetry of chromium(VI)

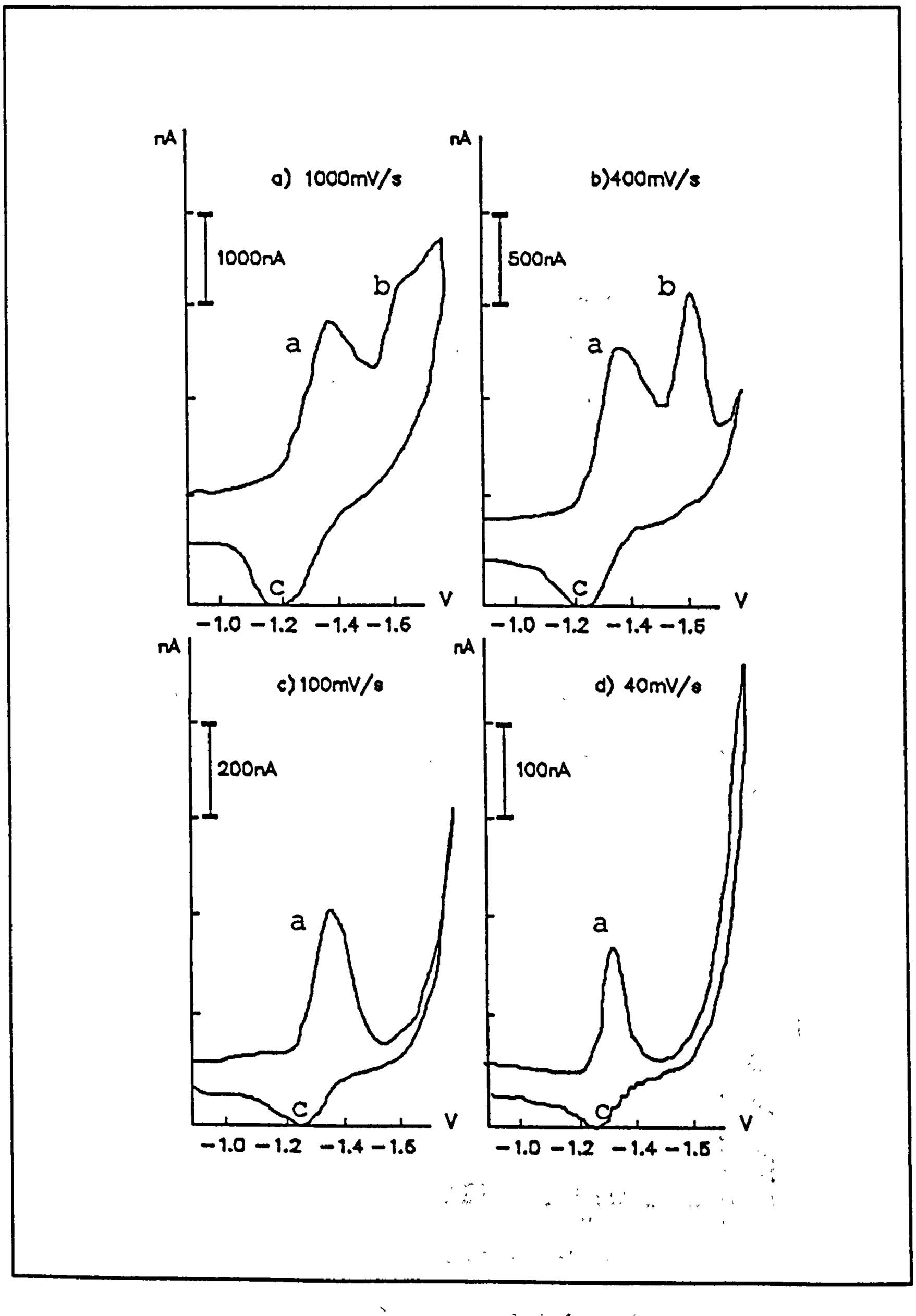


Figure 12 Comparison of the effect of scan rate on the cyclic voltammetry of chromium(III) and chromium(VI)

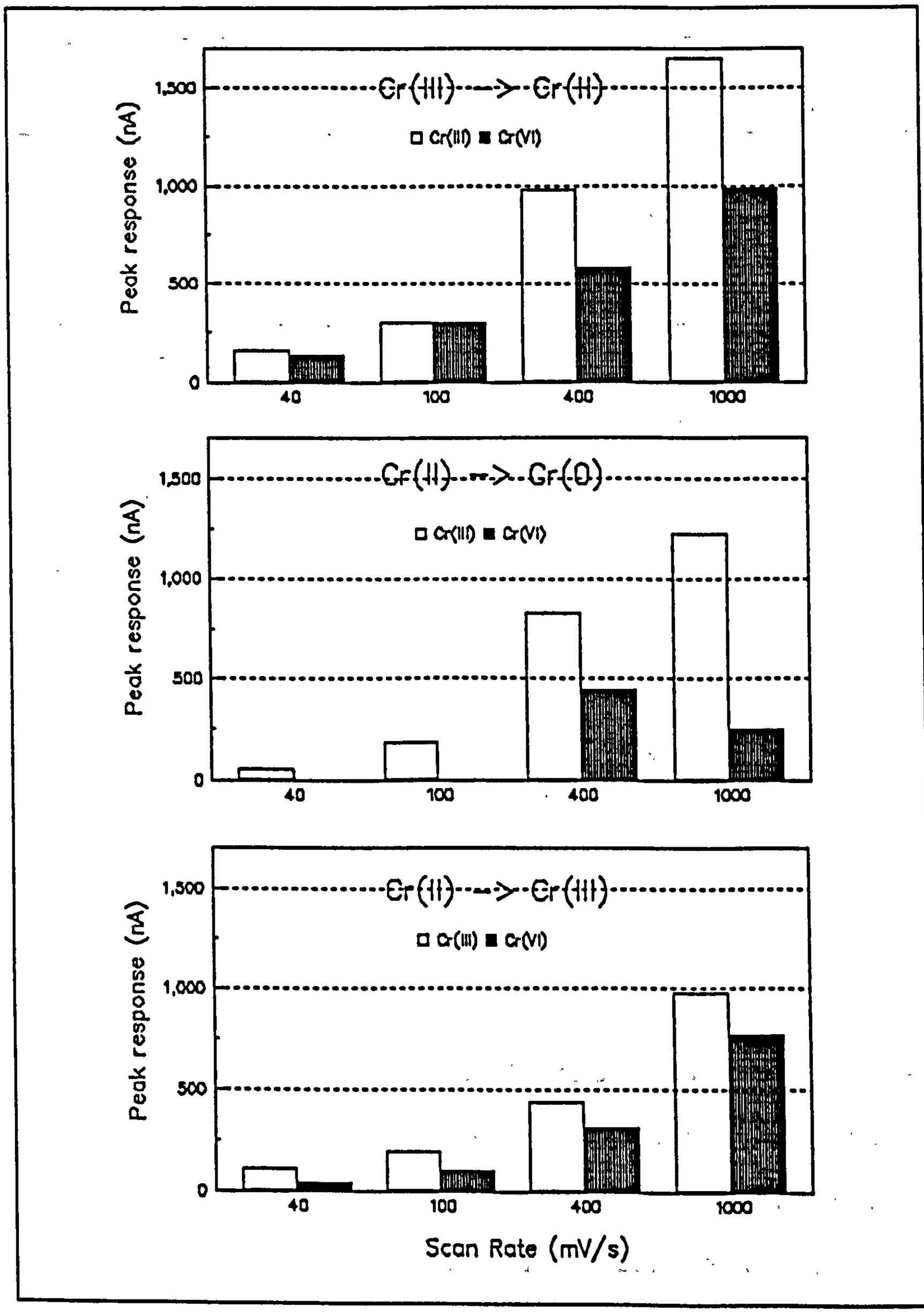


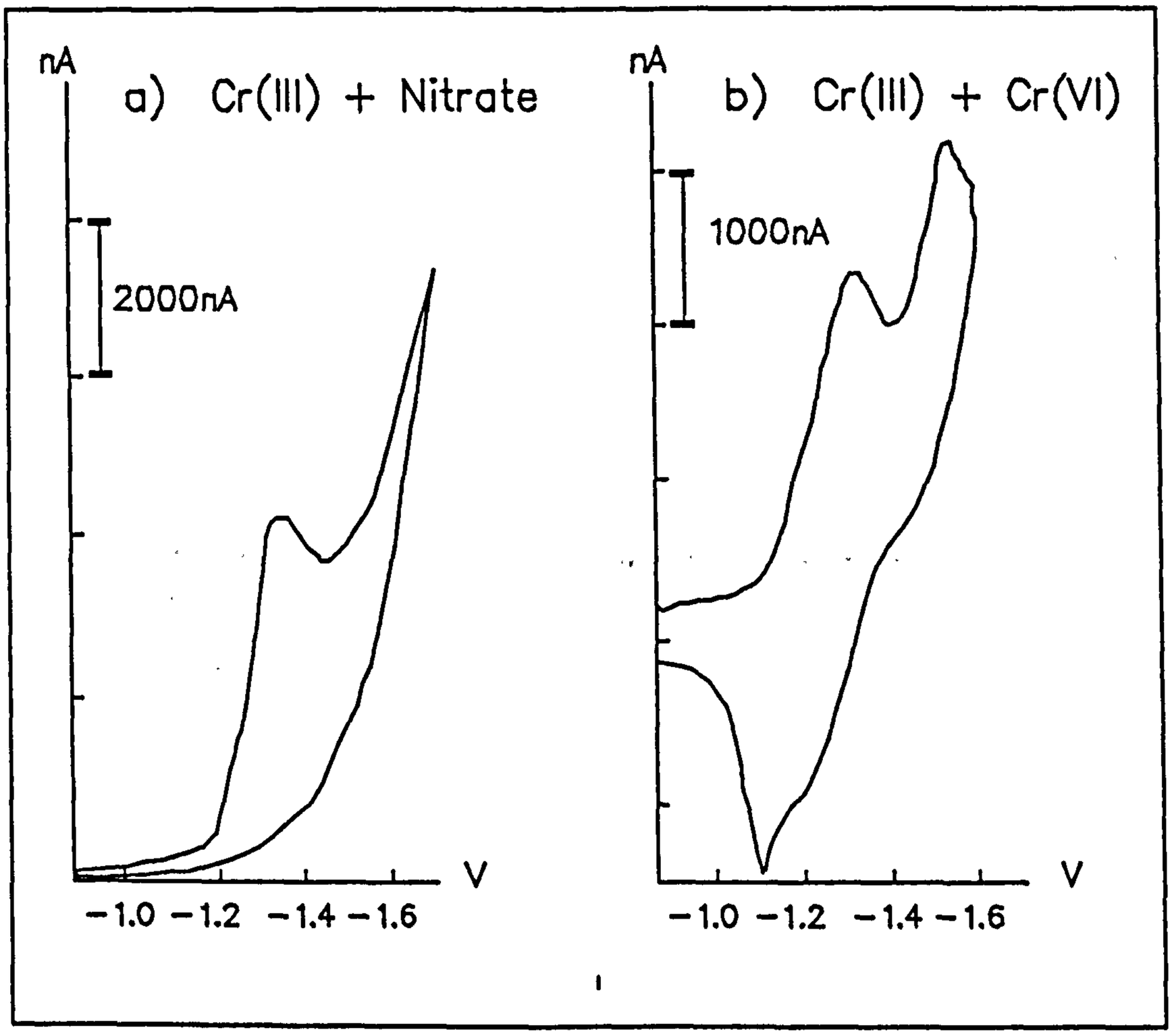
**Table 12 Effect of chromium(VI) and nitrate on the cyclic voltammetry of chromium(III)**

| CV Rate $mVs^{-1}$ | | Peak Current (nA) | | |
|---|---|---|---|---|
| | | Cr(3->2) (a) | Cr(2->3) (c) | Cr(2->0) (b) |
| 40 | Cr(III) | 161 | 114 | 54 |
| | + Cr(VI) | 238 | 157 | 213 |
| | + $NO_3$ | 377 | - | - |
| 100 | Cr(III) | 301 | 198 | 185 |
| | + Cr(VI) | 377 | 280 | 385 |
| | + $NO_3$ | 561 | - | - |
| 400 | Cr(III) | 980 | 445 | 829 |
| | + Cr(VI) | 760 | 1215 | 361 |
| | + $NO_3$ | 1136 | - | - |
| 1000 | Cr(III) | 1656 | 980 | 1226 |
| | + Cr(VI) | 1593 | 2750 | 318 |
| | + $NO_3$ | 1720 | - | - |

Unlike the addition of nitrate, which reduced the response of chromium to a single peak, peak a, that of chromium(VI) altered, but did not eliminate the other responses, peaks b and c, table 12, and figure 13b.

Further examination of the responses at varying CV scan rates showed that the second reduction peak, peak b, was approximately constant over the range examined. This may be due to two different mechanisms effecting the response. The first would be the same as with nitrate ions, *i.e.* due to the re-oxidation of the chromium(II)-DTPA complex, leading to a reduced response. At lower rates, while there is more time for this effect to take effect, there would also be time for chromium(VI) to diffuse into the

**Figure 13 Effect of oxidising agents on the chromium(III) cyclic voltammetric response**




boundary zone of the electrode, and then be reduced to chromium(II), and hence to chromium(0). This was supported by the observation that the peak increased with increasing concentrations of chromium(VI). These two effects obviously work in different directions, and on the evidence of this experiment, they approximately balance each other.

To test further this hypothesis, the ratio of the chromium(II) reduction peak to the chromium(III) reduction peak was checked at different CV scan rates. This ratio ought to have reduced at higher rates if diffusion was the

principal mechanism. In fact the reverse of this occurred, as shown in table 13, showing that the concentration of chromium(II) was reducing at the lower rates. This change in the chromium(II) concentration would be explained by either diffusion at the lower rates, or increased time for the chromium(VI) to oxidise the chromium(II) back to chromium(III), *i.e.* the reverse of the situation discussed above, and described in table 12.

**Table 13 Effect of chromium(VI) on the cyclic voltammetry of chromium(III) at different rates.**

| CV Rates ($mVs^{-1}$) | 40 | 100 | 400 | 1000 |
|---|---|---|---|---|
| Peak Ratio {Cr(2) -> Cr(3):Cr(3) -> Cr(2)} | | | | |
| Cr(III) | 0.71 | 0.66 | 0.45 | 0.59 |
| C(VI) | 0.33 | 0.35 | 0.55 | 0.78 |
| Cr(III) + Cr(VI) | 0.66 | 0.74 | 1.60 | 1.73 |
| Peak Ratio {Cr(2) -> Cr(0):Cr(3) -> Cr(2)} | | | | |
| Cr(III) | 0.33 | 0.61 | 0.85 | 0.74 |
| Cr(VI) | 0.00 | 0.00 | 0.78 | 0.25 |
| Cr(III) + Cr(VI) | 0.90 | 1.02 | 0.48 | 0.20 |

### 3.3.6 Effect of lead on chromium responses

The interference of chromium(VI) on chromium(III) scans needed to be removed, and ways of achieving this were investigated. One approach that appeared to offer a solution was the addition of a precipitation agent to remove the chromium(VI). Two elements with low chromate solubilities, lead and barium, were considered. The lead was successful in removing the interference, and further

enhanced the CV scans being obtained. This enhancement was mainly of the Cr(III) -> Cr(II) response, and not the later Cr(II) -> Cr(0) response, (table 14).

Blank experiments demonstrated that the enhancement could not be due to some reaction involving lead alone. One possibility was that as lead formed a Pb(0)-Hg amalgam at the plating potential, (-0.9V), ligand exchange could occur between chromium(III) and Pb-DTPA on the mercury drop. A further possibility was that the $Pb^{++}$ oxidized the chromium(II) back to chromium(III) in a similar manner to that of chromium(VI) or $NO_3^-$. This is supported by the oxidation potentials, shown below;

$Cr^{3+} + e \rightarrow Cr^{2+}$ -0.41

$Pb^{2+} + 2e \rightarrow Pb^{\circ}$ -0.126

**Table 14 Effect of lead on chromium(III) reduction.**

| | Peak Current (nA) | |
|---|---|---|
| | Cr(3 -> 2) | Cr(2 ->0) |
| Cr(III) | 1256 | 1190 |
| Cr(III) + Pb | 2553 | 1518 |
| Cr(III)&(VI) + Pb | 2257 | 1419 |

Evidence to support the former mechanism came from the observation that when the CV scan rate was varied, the ratio of the two reduction peaks changed in a similar manner to that of chromium(III) alone, (table 15), suggesting that the observed responses were being enhanced at the concentration stage, and not during the subsequent reactions.

Table 15 The effect of lead on the ratio of the chromium(III) reduction peaks, (Cr2->0/Cr3->2)

| | Cyclic Voltammetry Rates ($mVs^{-1}$) | | | |
|---|---|---|---|---|
| | 40 | 100 | 400 | 1000 |
| Cr(III) | 0.34 | 0.61 | 0.85 | 0.74 |
| Cr(III) + Pb | 0.33 | 0.48 | 0.66 | 0.73 |

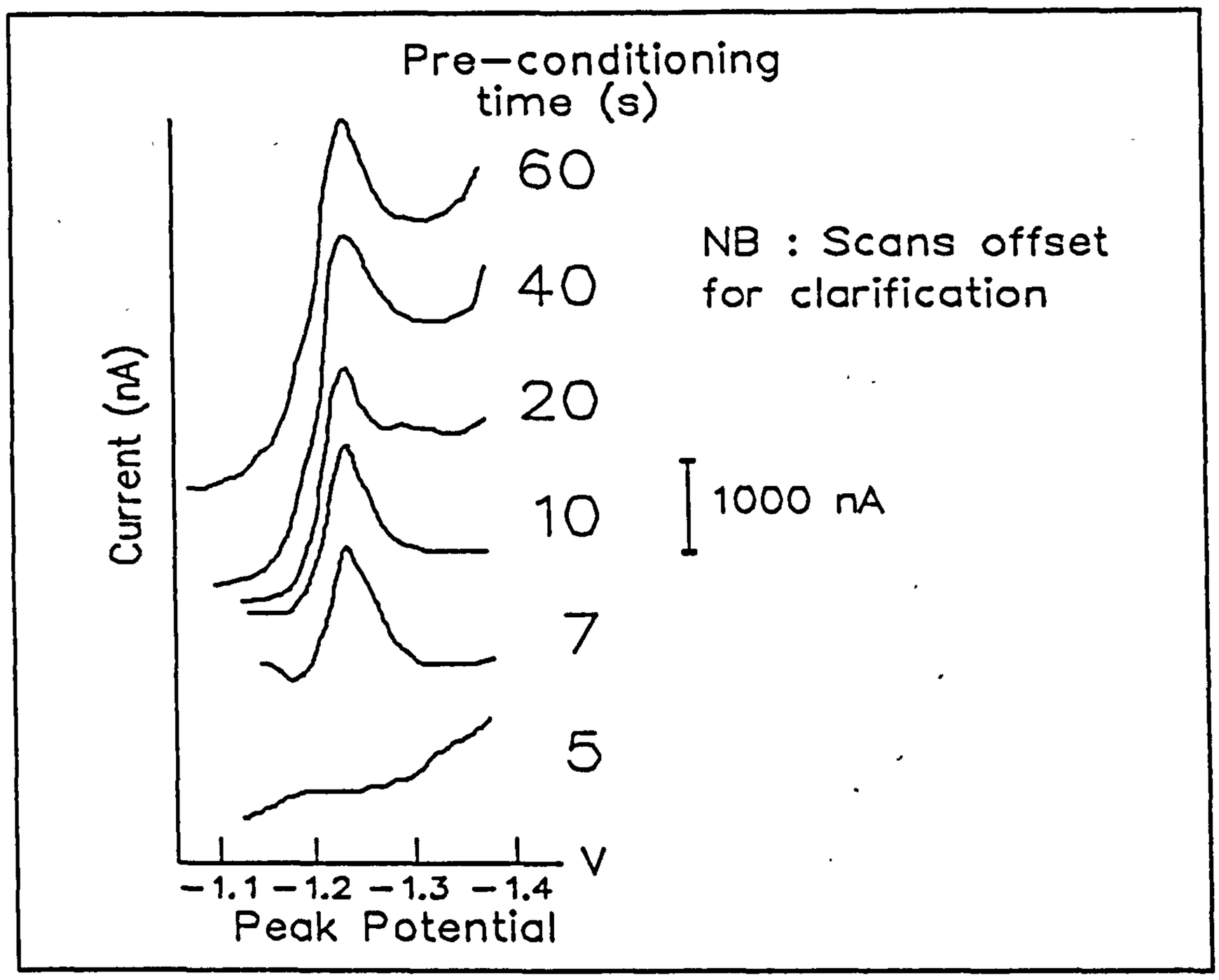
## 3.4 CYCLIC VOLTAMMETRY EXPERIMENTS IN BRITTON & ROBINSON, (B&R), BUFFER

One attempt to investigate an alternative buffering system that could allow the simultaneous determination of chromium(III) and chromium(VI), lead to the use of B&R buffer. Initial experiments, however, yielded unpredictable responses for chromium(III) and chromium(VI), leading to a lengthy examination of the variables involved.

Investigations into the age of the chromium solutions, effect of the stirrer or drop size showed that none of these were responsible for the variability. The time between extrusion of the mercury drop and application of the initial potential was then investigated. As may be seen in figure 14, at least 7 seconds was necessary for a consistent response to be obtained.

It is considered that this is necessary to allow the DTPA some time to coat the drop, before the other constituents

**Figure 14 Effect of pre-extrusion of mercury drop on chromium(VI) response in Britton & Robinson buffer**




of the buffer can compete for adsorption sites after the initial potential is applied. Evidence to support this can be drawn from the following observations :

a) reducing the concentration of the DTPA to one hundredth that of the chromium did not affect the production of a typical response;

b) an attempt was made to precondition the drop in a solution of DTPA, in the absence of the buffer, followed by media exchange to a cell containing buffer, chromium but no DTPA. The results indicated that a normal response was possible.

All the following experiments were thus carried out by extruding the mercury drop into the solution 10 seconds prior to applying the initial potential.

The chromium(III) response obtained in this buffering system was similar to that obtained in acetate, but reduced to approximately one tenth. This reduction was probably caused by the competition from the other ligands present in the B&R buffer, as noted above.

An initial examination was undertaken into the effect of changing pH, and adding nitrate to the chromium responses in B&R buffer. This showed that the response obtained for chromium(III) was similar to that obtained in acetate buffer alone, at pH 6.2. At pH 4.0, DTPA yielded a peak regardless of the chromium(III) concentration, while at pH 8.0, the response was poorly resolved, and quantification was very difficult. The effect of the nitrate as an oxidising agent was similar to that noticed in acetate buffer, except the overall response was lower, as noted above, and the background current higher, leading to a masking of the reduction peak.

The effect of altering the pH was confounded by several changes occurring together. In order to eliminate these factors, and to investigate the occurrence of a single large response for chromium(VI), it was decide that the constituents of B&R buffer, citric acid, barbitone, potassium phosphate and boric acid, would be added

separately to an acetate buffer system. The cyclic voltammetry of either chromium(III) or chromium(VI) at pH 6.2 or 8 was then be carried out.

### 3.4.1 Effect of boric acid on chromium(III) and (VI)

At pH 6.2, a typical scan was obtained for both chromium oxidation states, however, at pH 8 the Cr(III)->Cr(II) response was reduced while that of Cr(II)->Cr(0) was normal. As described in section 2.5.2.2, the major difference in the form of boric acid at these pH, is an increase from 0.1 to 6% of $B(OH)_4^-$. Whether this change has an impact on the electrochemical signal in the cell is doubtful. However, as there was little possibility of using the chromium(II) response for quantification it was decided not to investigate further this response.

### 3.4.2 Effect of citric acid on chromium(III) and (VI)

A typical scan was obtained for chromium(III) at pH 6.2, which was affected at pH 8, by the loss of the chromium(II) reduction peak. Chromium(VI) yielded a large single response, at a potential, equivalent to that for the reduction of chromium(III) to chromium(II). These facts may be explained by the ability of citric acid to behave as a reducing agent:

Thus the chromium(VI) was reduced in the electro-chemical cell, and as nascent chromium(III), was more likely to

react with DTPA, producing a large response. The absence of the following reaction Cr2->0 was similarly explained, as the chromium(II) was reduced chemically before the electro-chemical reaction, forming Cr(0), which is electro-chemically irreversible. This response was further investigated, and an analytical approach for the determination of chromium(VI) developed, see section 3.8

### 3.4.3 Effect of phosphate on chromium(III) and (VI)

At pH 6.2 the chromium(III) response was modified by the loss of the Cr2->0 peak, and that of Cr3->2 moved to a less negative ~~lower~~ potential. At pH 8 this response shifted back to its normal potential, but the Cr2->0 peak was still missing. These changes are not fully understood, but a preliminary investigation of the distribution of the chemical species in the cell, using MINEQL, suggested that the composition changed from 75% $H_2PO_4^-$ and 23% $HPO_4^-$ at pH 6.2, to 3% $H_2PO_4^-$ and 95% $HPO_4^-$ at pH 8.

### 3.4.4 Effect of barbitone on chromium(III) and (VI)

At pH 6.2, barbitone had no effect on the electro-chemistry of chromium(III) or chromium(VI). At pH 8, however, it would appear to totally complex chromium(III), yielding a CV curve very similar to that of a control sample. As may be seen from the data in section 2.5.2.5 the increase of unprotonated barbitone, from 1.6 % at pH 6.2 to 51% at pH 8 may explain this result. On the

basis of this data it is suggested that chromium(III) is complexed by unprotonated barbitone, and is electrochemically unreactive.

## 3.5 SQUARE WAVE VOLTAMMETRY INVESTIGATIONS

Having explored a number of important variables, using cyclic voltammetry, the development of an analytical method for environmental determination of chromium(III) and (VI) was begun. The method was to be based on that described by Golimowski *et al.*, (76), with two exceptions:

a) the stripping technique chosen was square wave voltammetry, for reasons which will be discussed below, and

b) the method would be used to determine chromium(III) only, chromium(VI) having been removed by addition of lead.

The principal gain in the use of square wave voltammetry lies in its use of faster scan rates. This yields two advantages, firstly an improvement in sensitivity, and secondly, faster analysis times. However, square wave voltammetry, also needs to be carefully investigated as it is more prone than other methods to electrode surface effects.

### 3.5.1 Theory of square wave voltammetry

In square wave voltammetry a symmetrical square wave is imposed on a staircase waveform, where the forward pulse of the square wave, is coincident with that of the staircase step. The reverse pulse, however, occurs halfway through the staircase step, figure 5b. The time for one staircase step, or a complete square wave cycle, is $\tau$ s, and the frequency in Hz is $1/\tau$. The nett height in the square wave pulse is the pulse height, mV, and the scan rate is calculated from

$$Scan\ Rate(mV/s) = \frac{E_{step}(mV)}{\tau(\text{sec})}$$

This means that with for example a frequency of 100 Hz, *i.e.* $\tau$ = 0.01 s, and a step size of 4 mV, the scan rate would be 400 mV $s^{-1}$, which is much faster than other pulse techniques.

The current in the cell is sampled on two occasions, once at the end of the forward pulse, and again at the end of the reverse pulse. By delaying the current measurements to the end of the charging period, charging currents are not measured. The difference between the two measurements is plotted versus the potential of the staircase. In effect this procedure means that square wave voltammetry obtains peaks for faradaic processes, with the peak height being directly proportional to the concentration of the responding ion in solution.

### 3.5.2 Experimental conditions

The operating parameters used were :

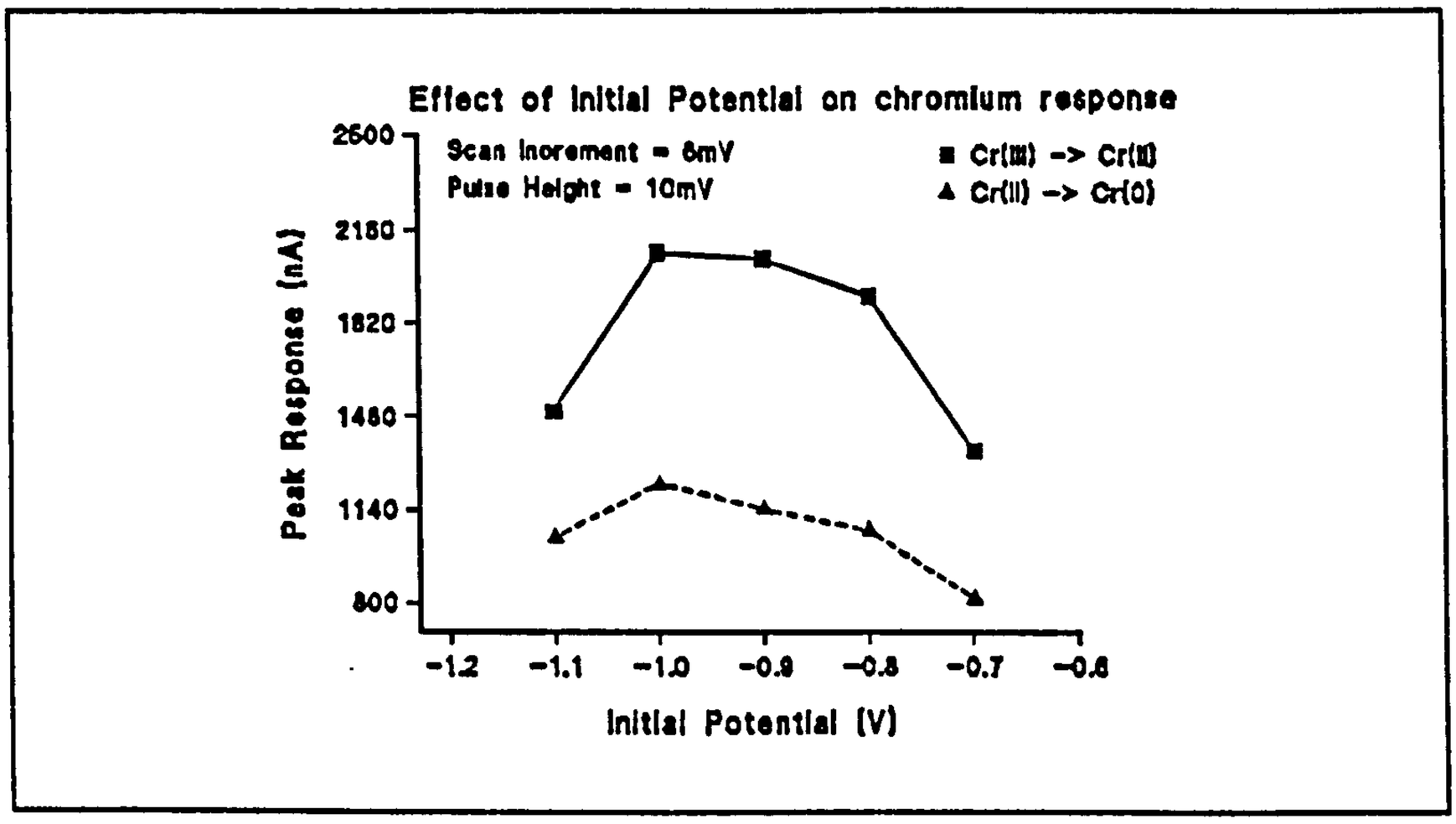
| | |
|---|---|
| Initial Potential | -0.9 V |
| Final Potential | -1.7 V |
| Gas Purge | 120 s |
| Deposition time | 30 s at -0.9 V |
| Equilibration time | 10 s |
| Frequency | 100 Hz, unless stated |
| Pulse Height | 20 mV, unless stated |
| Scan Increment | 6 mV, unless stated |

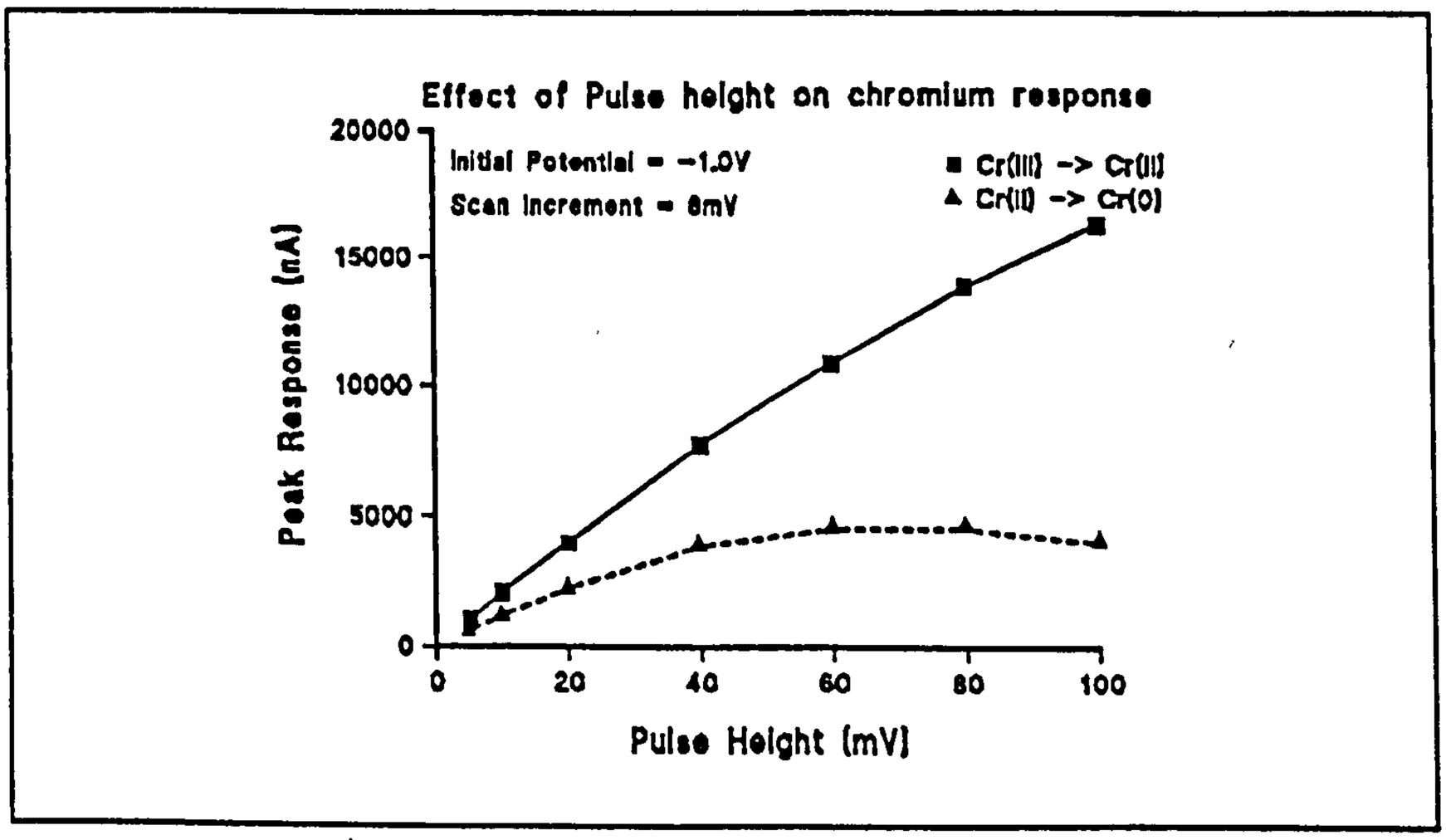
The electro-chemical cell was first filled with 10 ml of Milli-Q water, then 200 µl of $5x10^{-3}$ M DTPA added, followed by 100 µl of 1 M sodium acetate, and 500 µl of $5x10^{-3}$ M lead acetate. The cell was then analyzed, and chromium(III) or (VI) added.

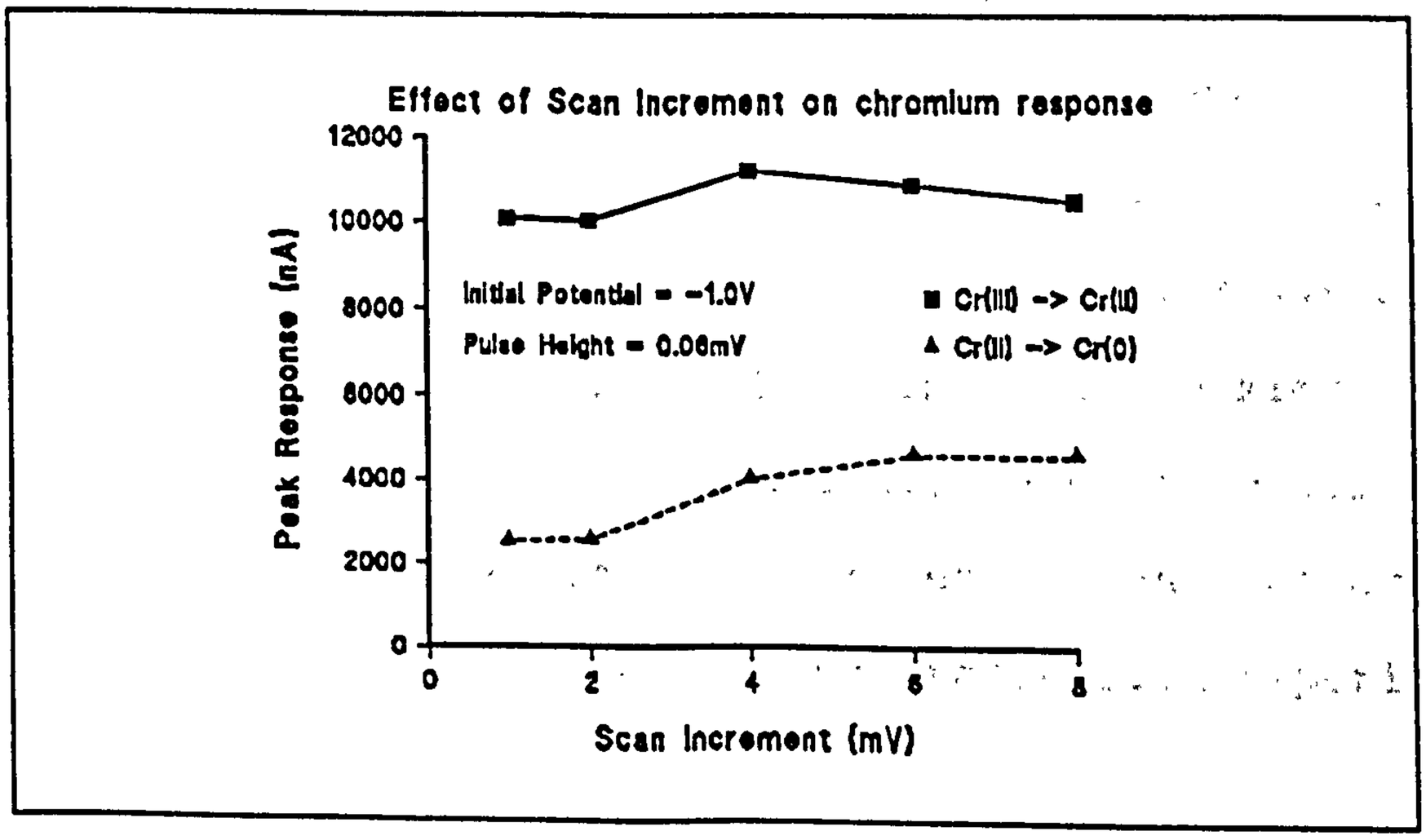
Using these conditions initial experiments investigated the effect of varying initial potential, pulse height and scan increment. The results of these investigations are shown in figure 15.

One of the possible advantages arising from the use of square wave voltammetry is the ability to separate differing responses, at the electrode, depending on their kinetics. In this system a large background interference was present, and an attempt was made, using simplex

Figure 15 Investigation into variables used in square voltammetry



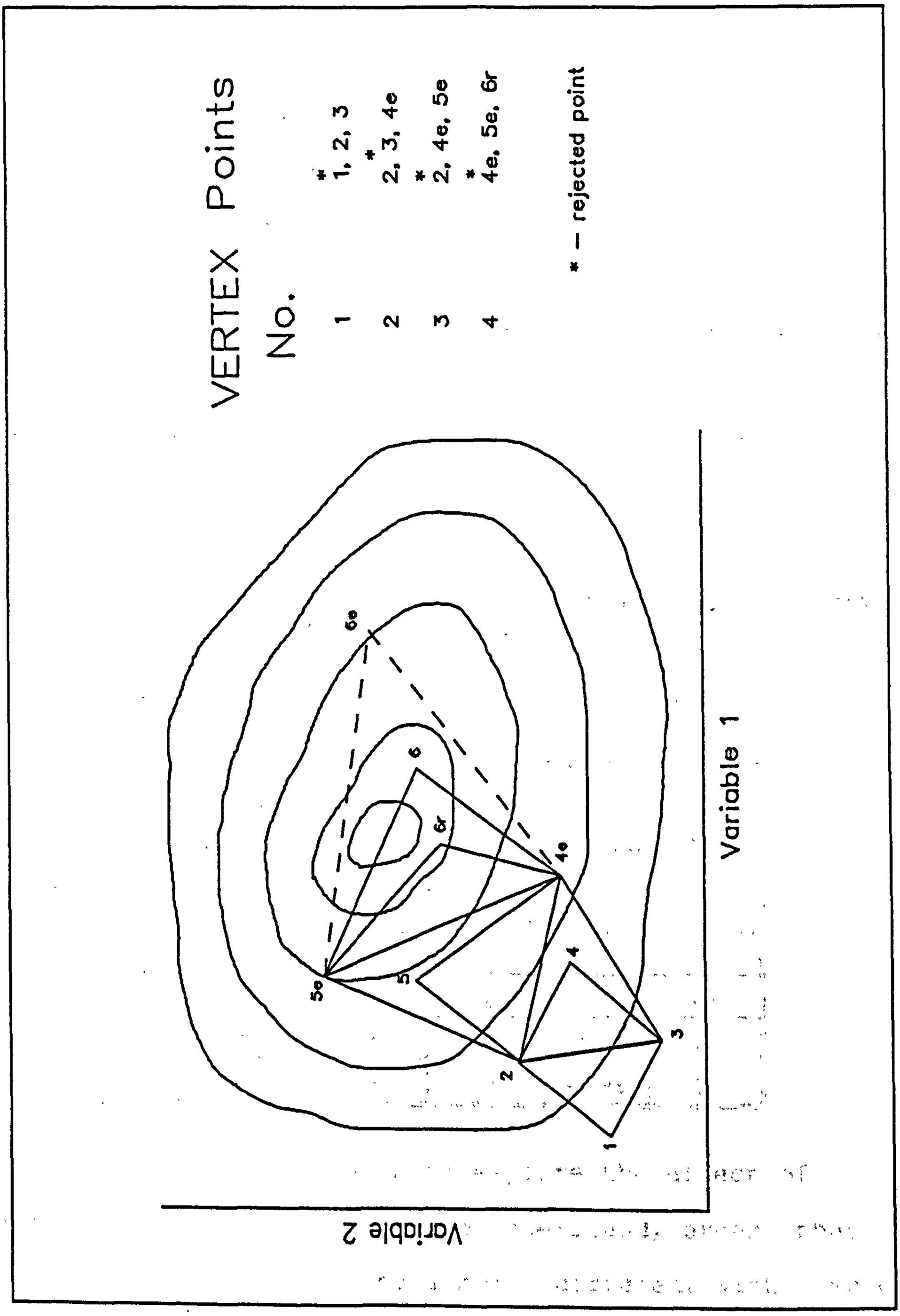



optimisation to reduce this interference with respect to the chromium(III) response.

The simplex optimisation technique was originally defined by Spendley *et al.*, (96), and later modified by Nelder and Mead, (97). The algorithm uses the data obtained during the optimisation experiment, to guide the direction of further experiments away from areas of low response. A simplex is defined as a geometric figure, having one more vertex than variables being explored. In the simplest case of two variables, the simplex is a triangle, and three experiments are set up, each corresponding to a point on the triangle. A number of simple rules then define the procedure to follow, and define the next experiment. A simple two variable simplex, and its progress is shown in figure 16.

The rules select the vertex with the worst response, and then reflect that vertex about the other points, hence defining a new point. When originally described by Spendley, the simplex was of a fixed size, this was a limitation, as the size of the simplex dictated the speed and precision of the search. This could mean finding only a local maximum, in cases where the simplex was set too small, or being unable to home in on the maximum, if set too large. Nelder and Mead overcame this by defining further rules enabling the simplex to contract or expand, depending on the success of the reflection experiment.

**Figure 16 Progress of a simplex experiment**



Simplex optimisation has been used widely in improving a number of analytical parameters, and particularly signal to background ratio, (98).

Two simplex programmes were used, both obtained from the University of Plymouth. These were essentially similar, the major difference being in their ease of use, and in the choice of initial conditions, and the rate of change imposed on the variables. Despite these differences, the simplex selected final conditions which were reasonably similar for all the variables, with the exception of the deposition time, table 16.

**Table 16 Final vertices of simplex optimisation of square wave voltammetry of chromium(III)**

| Vertex | Freq. (hz) | Pulse Ht. (mV) | Dep. Time (s) | Scan Inc. (mV) | Init. Pot. (V) | Resp. (nA) |
|---|---|---|---|---|---|---|
| Simplex 1 | | | | | | |
| 13 | 110 | 0.16 | 150 | 8 | -0.77 | 4266 |
| 15 | 95 | 0.14 | 133 | 8 | -0.92 | 3012 |
| 16 | 117 | 0.05 | 160 | 10 | -0.86 | 5050 |
| Simplex 2 | | | | | | |
| 11 | 74 | 0.15 | 36 | 8 | -0.85 | 2629 |
| 13 | 93 | 0.25 | 42 | 10 | -0.80 | 4745 |
| 16 | 105 | 0.12 | 37 | 10 | -0.88 | 4080 |

The reluctance of simplex 2 to explore the effect of deposition time is difficult to understand, except that the rules for enlarging the simplex differed, with simplex 1 expanding at a greater rate than simplex 2.

The principal findings of these experiments, however, may be summarised as follows. The parameters needed for a square wave method to give high signal to background ratio, are a frequency of 100±20 Hz, and a scan increment of 8±2 mV. Increasing the deposition time, and having an initial potential of -0.8±0.2 V, will also improve sensitivity, but responses to interferences also increase.

This method was not developed any further, as interferences were experienced in the system being used, relating to the addition of nitrate and lead, which proved difficult to remove. While the former was identified as contamination in the solution, and could have been eliminated, the latter was not. Contamination of the solutions was eliminated, using different sources, and purer chemicals. Other possibilities including the electrodes were all eliminated by, for example, substitution.

It was subsequently decided to investigate the interference, which had been identified as being related to the addition of lead.

## 3.6 INVESTIGATIONS INTO THE INTERFERENCE CAUSED BY LEAD

### 3.6.1 Initial investigations

Initially it was confirmed that the response was caused by the addition of lead. The presence of DTPA was essential

for the response to be seen, and nitrate enhanced the response in a similar manner to that of chromium. Using ultra-pure lead, changing the mercury in the HMDE reservoir, and using a new mercury capillary did not eliminate the response.

### 3.6.2 Electrochemical behaviour of lead interference

As may be seen in table 17, increasing the lead concentration led to an increase in the interference. However, beyond $2x10^{-4}$ M there was no further increase in the response, although comparison with chromium(III) shows that the chromium(III) signal was not saturated at that level. In a separate experiment, it was also noticed that the response due to lead, was only evident at concentrations above $5x10^{-5}$ M.

Investigations were then carried out into the effect of the deposition time on the interference. These showed that although the response due to chromium(III) and (VI) increased with increasing deposition time, that due to the lead did not, table 18.

Finally the impact of changing the initial potential for the deposition was considered, table 19. As expected there was an increase in the chromium response with increasing initial potential until above -1.0 V, when the response declined. The lead response behaved in a similar manner, except that there was no decline in the response

**Table 17 Comparison of lead and chromium(III) signals with increasing concentration.**

| Lead | | Chromium (VI) | |
|---|---|---|---|
| Conc.($x10^{-4}$M) | Response (nA) | Conc.($x10^{-6}$M) | Response (nA) |
| 1 | 21,31 | 1 | 219,221 |
| 2 | 54,53 | 2 | 330,267 |
| 3 | 47,50 | 4 | 1040,822 |
| 4 | 51,64 | 6 | 1574,1429 |
| 5 | 59,66 | 8 | 2157,1984 |
| 6 | 48,55 | 10 | 2014,2419 |

**Table 18 Effect of deposition time on lead response and chromium responses (nA)**

| Deposition Time (s) | Lead ($2.4x10^{-4}$M) | Chromium(III) ($1x10^{-6}$M) | Chromium(VI) ($1x10^{-6}$M) |
|---|---|---|---|
| 30 | 140,138 | 37 | 255 |
| 60 | 137,140 | 44 | 270 |
| 120 | 131,128 | 55 | 299 |
| 180 | - | 59 | 330 |

**Table 19 Effect of initial potential on lead and chromium responses (nA)**

| Initial Potential (V) | Lead ($2.4x10^{-4}$M) | Chromium(III) ($1x10^{-6}$M) |
|---|---|---|
| -0.5 | 77,66 | 42,54 |
| -0.7 | 67,74 | 40,58 |
| -0.9 | 78,67 | 50,66 |
| -1.0 | 94,81 | 53,75 |
| -1.05 | 90,88 | 0,0 |

above -1.0 V.

As a result of these investigations it was decided that the response was due to a chemical reaction involving the

lead adsorbed on the mercury drop. Of the metals with electro-chemical potentials in the region being investigated, only iron was found when mercury from the HMDE was analyzed by ICP-OES. Apart from iron at 30 mg $kg^{-1}$, all the other metals considered, chromium, zinc, indium, nickel and vanadium, were present at less than the detection limit, <1 mg $kg^{-1}$, of the ICP-OES.

The possibility was that lead was involved in a displacement reaction with iron in the mercury drop. Thus lead formed a complex with DTPA preconcentrated on the mercury drop. The iron then displaced the lead, forming Fe(III)-DTPA. When the potential was raised, iron(III) was reduced, in a similar manner to that of chromium, to iron(II). Nitrate ions would catalyse this reaction, in a like manner to that of the chromium response.

The principle of displacement of lead in the determination of iron was described by Berge and Drecher, (99), who used an EDTA complex.

Although the addition of iron directly to the cell media did not lead to a significant response, this was not totally unexpected as the response noted by Berge and Drescher was insensitive, to greater than $1x10^{-5}$ M of iron. In the system under investigation, however, iron would be present within the drop, and effectively preconcentrated, which would thus lead to a much improved response.

As well as the problems discussed above, a high background current in the system, which proved to be difficult to lower, without reducing the sensitivity, was also experienced. It was therefore decided to use the same basic system as that described by Golimowski *et al.*, except that the method would be based on total, chromium(VI) and chromium(III), determination, and a separate chromium(VI) determination.

## 3.7 DEVELOPMENT OF SPECIATION AND MEDIA EXCHANGE SYSTEM

### 3.7.1 Initial investigations into the effect of electrochemical variables on chromium(III) and chromium(VI)

Prior to the development of a system using a flow cell and media exchange, the analytical performance of the static system was determined. The experimental conditions used were;

| | |
|---|---|
| Initial Potential | -1.0 V |
| Final Potential | -1.5 V |
| Purge time | 120 s |
| Deposition time | 30 s at -1.0 V |
| Drop/step time | 0.2 s |
| Scan increment | 8 mV |
| Scan Rate | 30 mV $s^{-1}$ |
| Equilibration time | 10 s |

100 ml of a base solution was prepared containing 0.05 M DTPA, (1.96 g), 5 M sodium nitrate, (41.6 g), and 0.2 M sodium acetate, (1.64 g), pH adjusted to 6.2 by appropriate addition of acetic acid or sodium hydroxide.

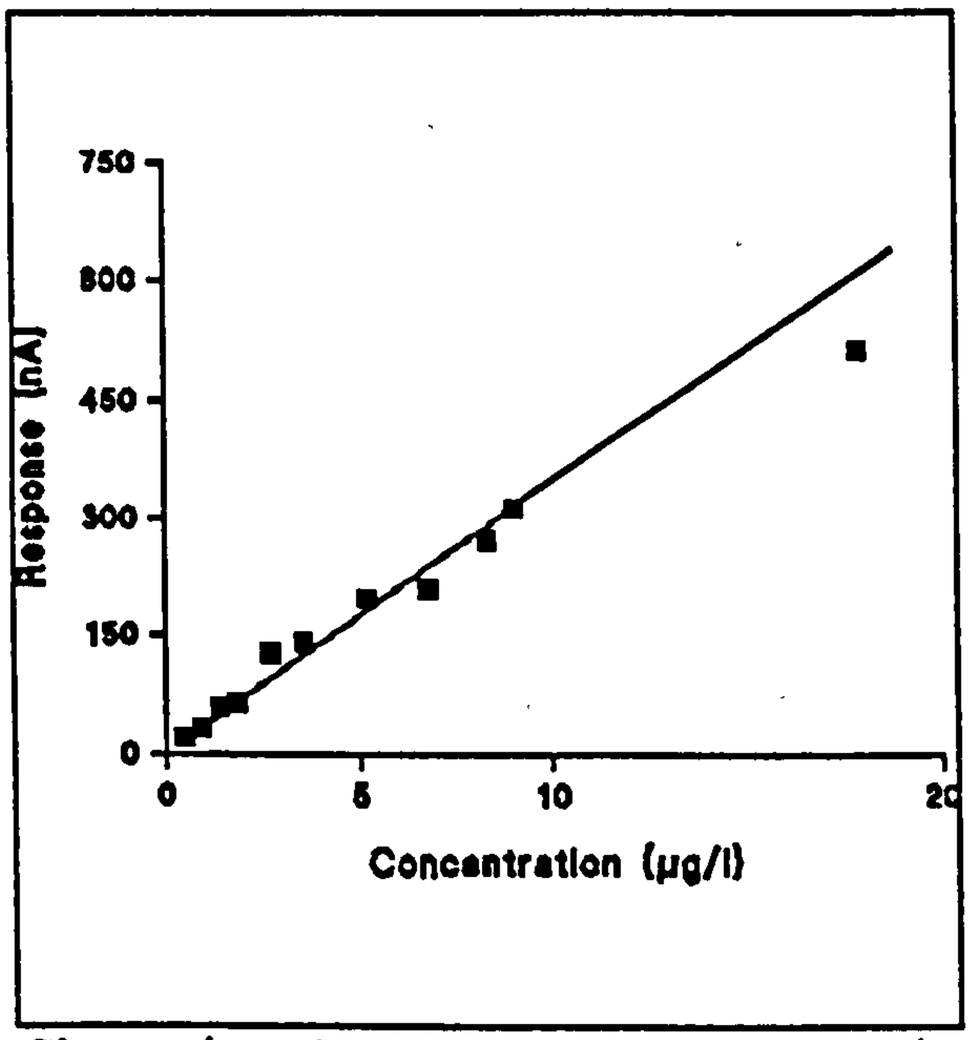
Chromium (III) and (VI) solutions, 1 $mgl^{-1}$, were prepared from 100 $mgl^{-1}$ stocks, made up by weight from chromic chloride, (51.2 mg of $CrCl_3.6H_2O$ in 100 ml), and potassium chromate, (37.3 mg of $K_2CrO_4$ in 100 ml) respectively. Aliquots of these solutions were then added to a cell containing 1 ml of base solution added to 9 ml of Milli-Q water.

Figures 17a and 17b show the responses obtained from these experiments, demonstrating the response to be linear over the range tested, with the sensitivity of the chromium(VI) response being 0.0196 $\mu g\ nA^{-1}$, and the chromium(III) response 0.0277 $\mu g\ nA^{-1}$. The ratio of these two responses, 1.4, compared favourably with that reported previously by Zarebski, (70), who also obtained a ratio of 1.4, while Torrance and Gatford, (74), obtained ratios of 1.2 and 1.4.

Based on the reproducibility of the lowest standard, the detection limit was calculated, using $3\sigma_{n-1}$. This gave a detection limit of 0.4 $\mu gl^{-1}$ for chromium(VI), and 0.6 $\mu gl^{-1}$ for chromium(III). These are higher than those reported by Boussemart, Golimowski, and Scholz, and co-workers, (25, 71, 73), whose detection limits for total chromium

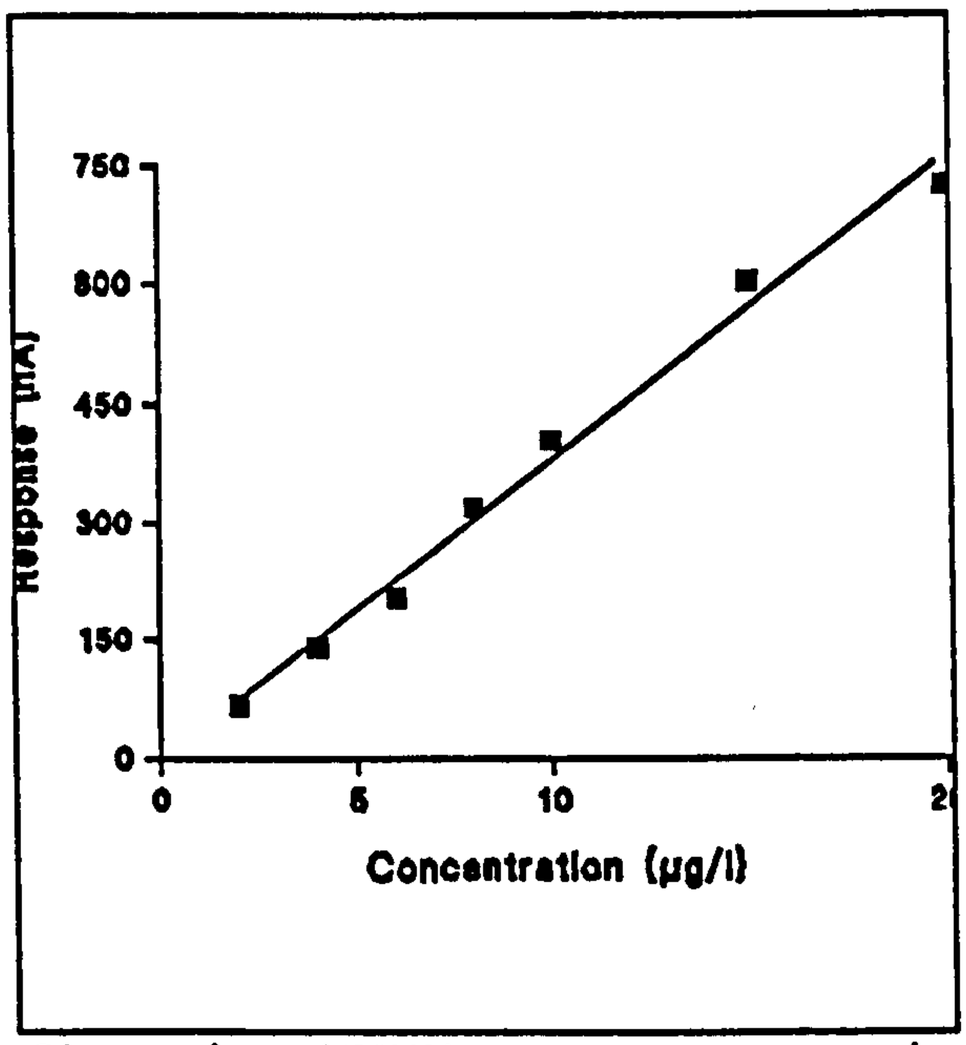
**Figure 17 Calibration curve for chromium(III) and chromium(VI) in static and flow systems**

Figure 17a



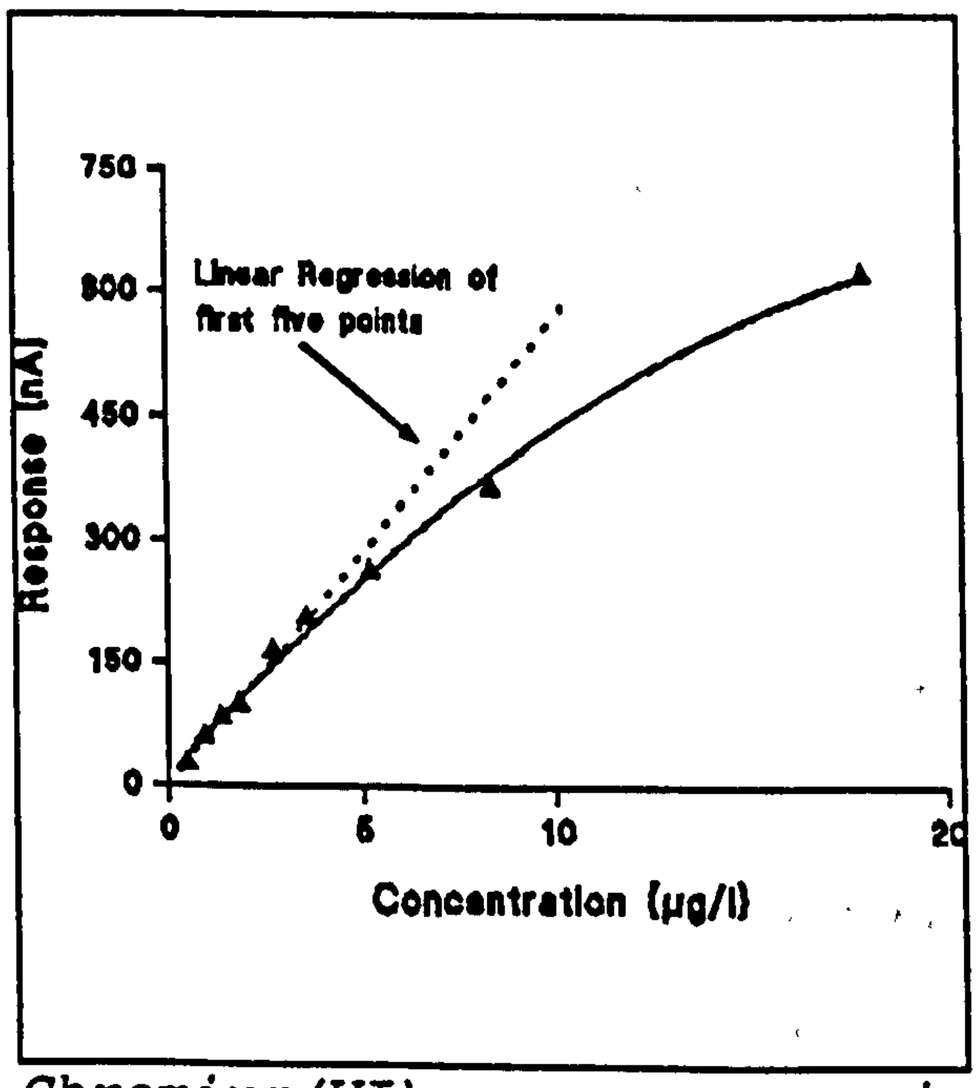

*Chromium(III) response in static cell*

Figure 17c



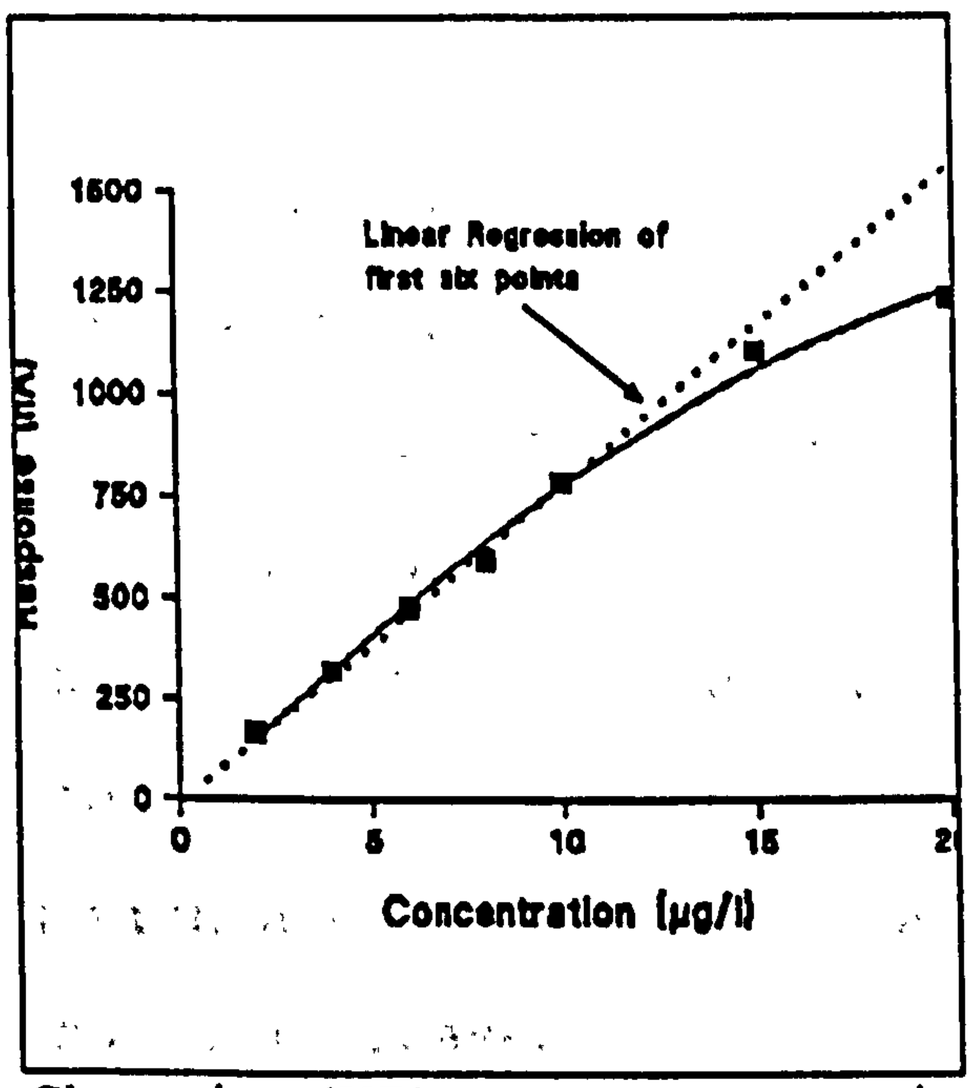

*Chromium(III) response in flowing cell*

Figure 17b




*Chromium(VI) response in static cell*

Figure 17d




*Chromium(VI) response in flowing cell*

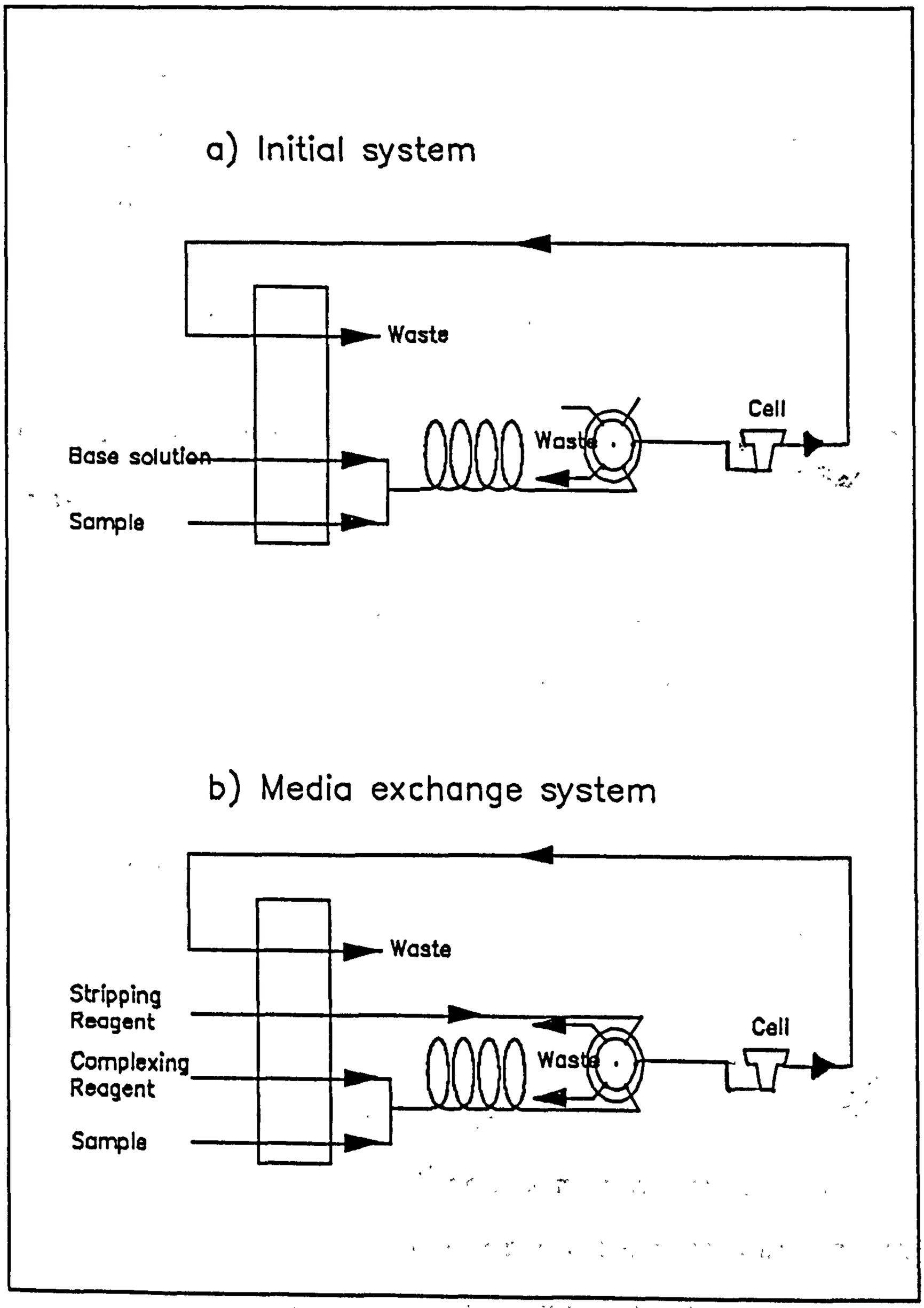
were 0.006 to 0.02 $\mu g l^{-1}$, but these were based on deposition times of 2 minutes or more, while only 30 seconds was used in this study.

Having established the analytical characteristics of the system under investigation, in static conditions, a flowing system was then constructed. The essential elements of this system are shown in figure 18a.

Initial experiments examined the characteristics of a simple flowing system, in which the sample was mixed in line with a degassed base solution. The ratio of sample to buffer was set at approximately 1:10, similar to that in the static method.

The analytical characterisation of this system was then performed, (figures 17c and 17d). The sensitivity for chromium(VI) and (III) was 0.0134 $\mu g\ nA^{-1}$ and 0.0281 $\mu g\ nA^{-1}$ respectively. While the detection limits, again based on $3\sigma_{n-1}$ for the lowest standard, were 0.4 and 1.5 $\mu g l^{-1}$. The main point of interest in these figures, was the altered ratio of chromium(VI):(III) sensitivity, due to an increase in the sensitivity of the method to chromium(VI). There were two possible explanations of this finding; either chromium(VI) was reacting with a constituent of the buffer in the mixing coil, leading to increased complexation of reactive chromium; or the ratio of base solution to sample was different to that in the static cell, and the impact was greater on chromium(VI) than

**Figure 18 Flowing configurations for the determination of chromium**



chromium(III). Changing the flow rates of the sample and base solution had no effect on the sensitivity of the method, however, by altering the base solution:sample ratio the sensitivities of the two chromium species were altered, along with their ratio. As reducing the base solution:sample ratio led to increased sensitivity it was decided to investigate the impact of the differing base solution constituents on the chromium responses.

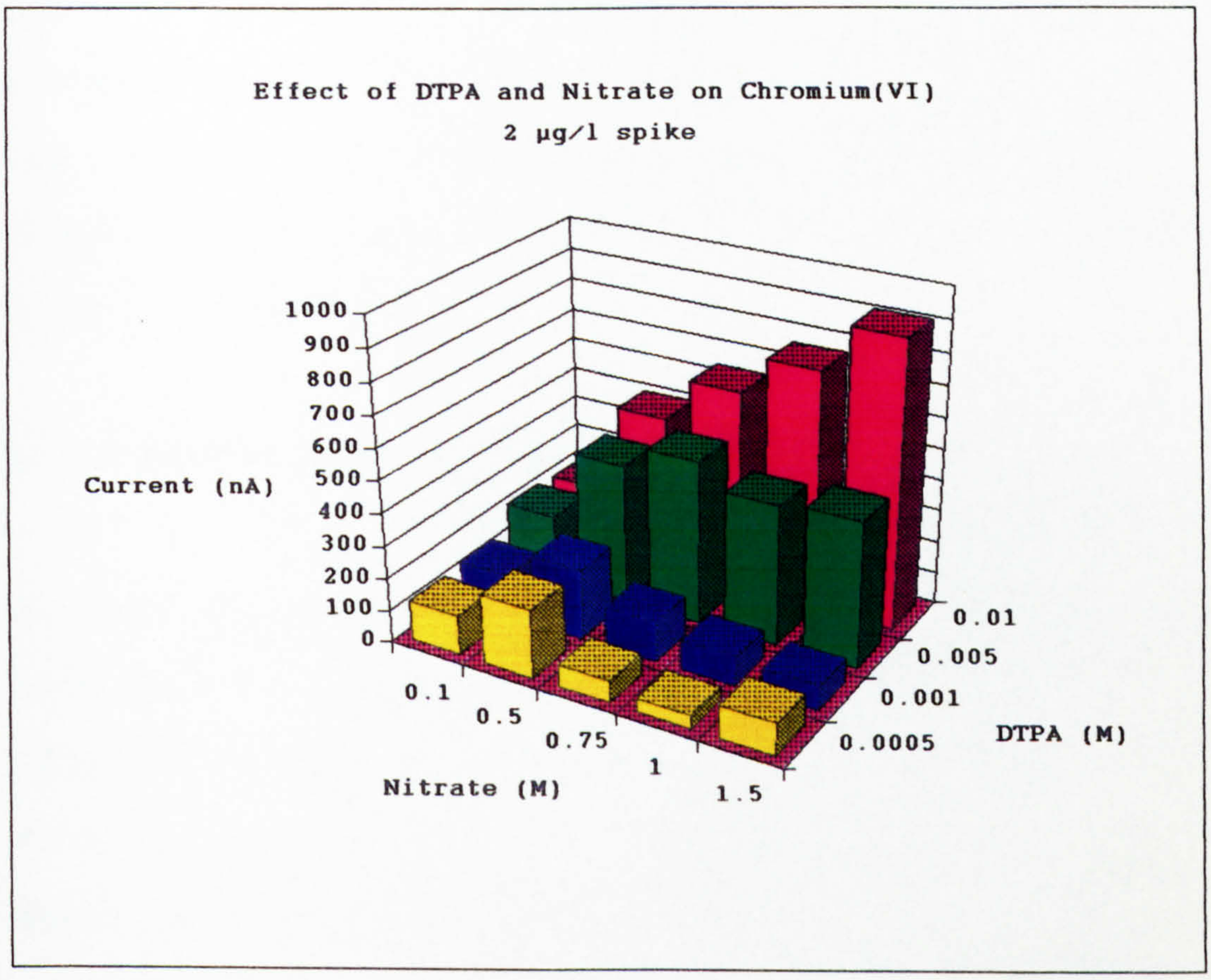
### 3.7.2 Effect of DTPA and nitrate on chromium(III) and (VI) responses

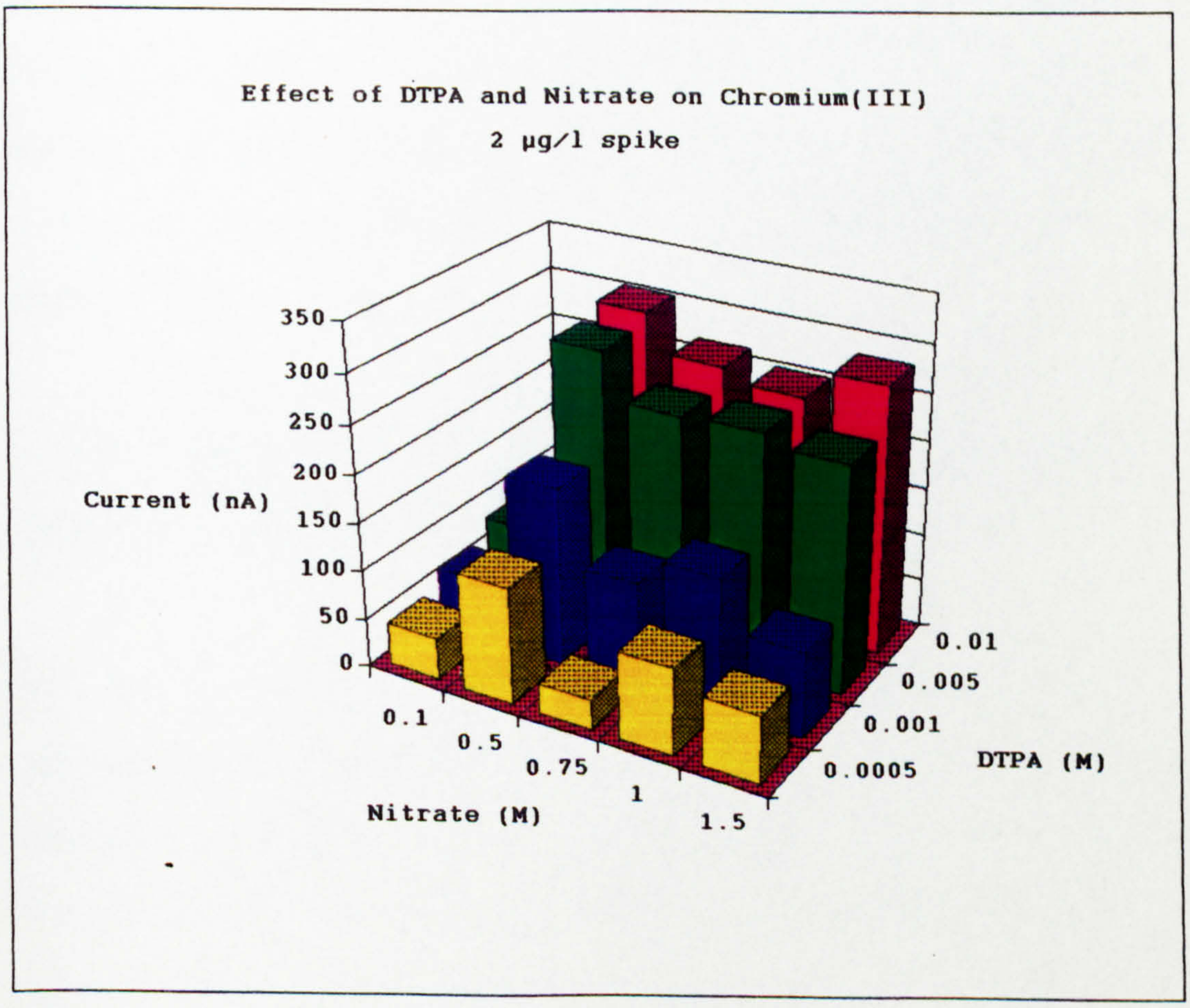
To evaluate the impact of changing these two constituents of the base solution, on the chromium responses, a multi-factor experiment was carried out. The concentration of nitrate was varied from 0.1 to 1.5 M, while that of DTPA ranged from 0.0005 to 0.01 M.The results from this experiment are shown in figure 19, for chromium(III) and (VI).

The data show that for chromium(III) and (VI), at low concentrations of DTPA, the optimum concentration of nitrate ion is 0.5 M. At higher concentrations of DTPA, changes in the concentration of the nitrate ion appeared to have no effect above the minimum concentration used of 0.1 M $NO_3^-$.

The concentration of DTPA needed to be 0.005 M or higher, for optimum sensitivity.

**Figure 19 Effect of DTPA and Nitrate on the Chromium(III) and (VI) response**



Clearly this should be explored more extensively, to enable a better explanation of the responses described above. The probable explanation for the nitrate effect is a limitation in the extent to which nitrate may catalyse the chromium(III) reduction. Thus the higher DTPA concentrations have no effect as the response is maximised by the nitrate ion.

### 3.7.3 Development of media exchange system

The system was set up as shown in figure 18b, and initial experiments demonstrated that the system yielded comparative data to that of the previous flowing system. As discussed in section 2, the optimum pH for the determination of chromium in estuarine waters was considered to be approximately pH 5. With a media exchange system, it was possible to examine separately the effect of pH on the two steps in stripping voltammetry, *i.e.* the deposition step, and the stripping step, this was undertaken.

#### 3.7.3.1 Effect of pH on complexation of chromium(III) and (VI) in fresh and seawater

The pH of the complexing reagent was set at 6.2 or 5, by appropriate addition of acetic acid or sodium hydroxide. 500 ml of freshwater or seawater was then spiked with chromium(III) or (VI) at 8 $\mu g l^{-1}$. Comparison of the appropriate data in table 20, shows that there is a slight

but significant difference between the complexation at pH 6.2 and 5.

**Table 20 Effect of pH of complexation and stripping streams on chromium(III) and (VI) responses**

| Water | pH of Complex. step | pH of Stripping step | Response (nA) |
|---|---|---|---|
| Chromium(III) @ 8 $\mu g l^{-1}$ | | | |
| Fresh | 5.0 | 5.0 | 22030, 15980, 20970 |
| | 6.2 | 5.0 | 20050, 21850, 14900 |
| | 5.0 | 6.2 | 3063, 2065, 2141 |
| | 6.2 | 6.2 | 1292, 1420, 1519 |
| Sea | 5.0 | 5.0 | 13000, 10360, 10830 |
| | 6.2 | 5.0 | 4390, 4850, 3305 |
| | 5.0 | 6.2 | 2289, 1841, 4810 |
| | 6.2 | 6.2 | 1618, 2016, 1660 |
| Chromium(VI) @ 8 $\mu g l^{-1}$ | | | |
| Fresh | 5.0 | 5.0 | 21490, 23080,21990 |
| | 6.2 | 5.0 | 16830, 22690, 18040 |
| | 5.0 | 6.2 | 3079, 3283, 1879 |
| | 6.2 | 6.2 | 1730, 1312, 1726 |
| Sea | 5.0 | 5.0 | 17740, 19100, 19500 |
| | 6.2 | 5.0 | 14970, 16160, 17560 |
| | 5.0 | 6.2 | 2149, 4076, 1969 |
| | 6.2 | 6.2 | 1990, 1762, 2087 |

### 3.7.3.2 Effect of pH on stripping signal of chromium(III) and (VI) in fresh and seawater

Using the same system and approach, the pH of the stripping stage was altered, and the signal obtained at pH 5.2 and 6. The data is shown in table 20, which shows a significant enhancement of the signal at pH 5.2.

While it was anticipated that the pH of the complexation step would be critical to the sensitivity of the method, because of the effect of pH on DTPA, it was not anticipated that the same would be true in the stripping stage.

It would seem from this data that the stripping signal due to the reduction of chromium(III) to (II), is enhanced by the hydrogen ion. Unfortunately lack of time meant that explanation of the effect of lower pH on the signal could not be undertaken, but it is possible that the signal could be further enhanced. It is a recommendation of this study that a full investigation of the effect of pH on the stripping signal be undertaken, using the media exchange system.

### 3.7.3.3 The effect of surfactants on the electrochemical signals of chromium(III) and (VI)

A brief evaluation demonstrated that naturally occurring surface active materials, as modelled by Triton X-100, still affected the response of chromium in the flowing cell. Thus at 1 $mgl^{-1}$ of Triton X-100, the signal from chromium(III) had been reduced by approximately 20%. This confirmed the report of Boussemart *et al.*, (25), although as predicted in that study the effect was lower at shorter deposition times. The effect was thought to be sufficiently small, given the likely concentration of such materials in the Tees that no further changes to method

were adopted to further reduce the effect, *e.g.* by UV irradiation of the samples.

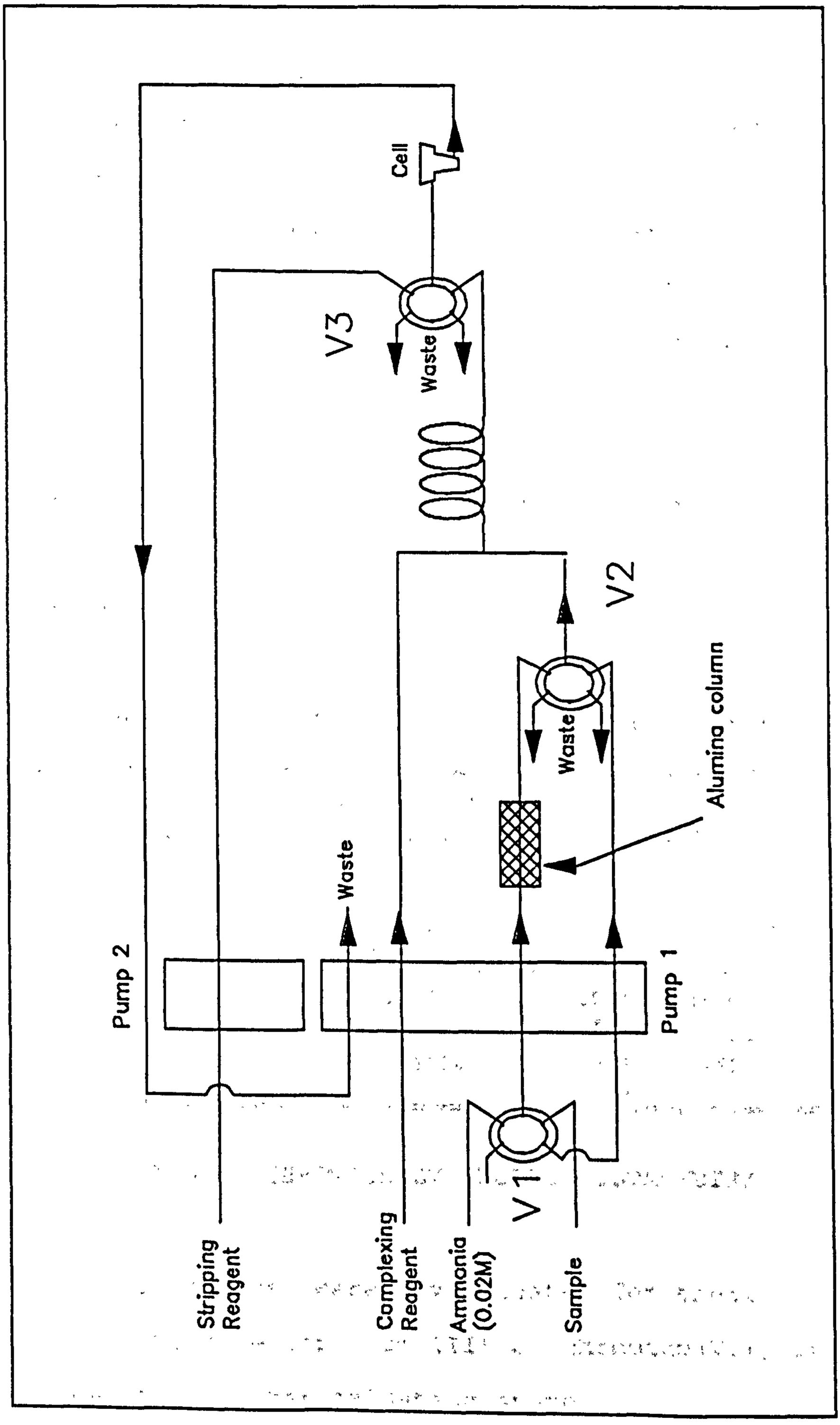
### 3.7.3.4 Introduction of an alumina column for the determination of chromium(VI)

In order to selectively determine chromium(VI) it was decided to pass the sample through basic alumina. This has been used by Cox *et al.*, (100, 101), when they used an acidic column to trap chromium(VI), prior to analysis by ICP-OES, and in a further study to preconcentrate chromium(III) on a basic column, prior to analysis by ICP-OES. In this study it was decided to remove the chromium(III) after determination of total chromium, and thus determine chromium(VI) directly, and chromium(III) by difference. Given the relative sensitivities of the two oxidation states in the electrochemical method, and the presumed higher level of chromium(VI) in the environment this was considered the most sensible approach.

A 4 cm by 0.2 mm id glass column was packed with activated alumina, and plugged at each end with glass wool. The alumina was obtained from BDH Chemicals, Poole, as Brockman Grade 1, basic form, particle size 75 - 120 µm.

The system described is shown in figure 20, which is basically the media exchange system with the addition of an ammonia stream to recondition the column after each sample. Using this system samples of chromium(III) and

**Figure 20 Media exchange system for the determination of chromium in estuarine waters**



(VI) were analysed, and the results are summarised in table 21.

The effect of the alumina column on chromium(III) is clear in table 21, as the response in freshwater is removed, while that in seawater is considerably reduced. Presumably the signal remaining represents a small concentration of chromium(VI) in the seawater, which using the response above would be approximately 0.5 $\mu g l^{-1}$.

The chromium(VI) response showed a slight reduction when passing through the alumina column. This was found to be consistent, and would need to be taken in to account when determining the concentrations of chromium(VI).

**Table 21 Determination of total chromium and chromium(VI)**

| Sample Description | Media | Total chromium (nA) | Cr(VI) (nA) |
|---|---|---|---|
| 5 $\mu g l^{-1}$ Cr(III) | Freshwater | 15560, 8710, 19180 | 0,0,159 |
| 5 $\mu g l^{-1}$ Cr(III) | Seawater | 5000, 4750, 10590 | 1538, 1992, 5320 |
| 5 $\mu g l^{-1}$ Cr(VI) | Freshwater | 23290, 27550, 26530 | 20570, 22870, 24530 |
| 5 $\mu g l^{-1}$ Cr(VI) | Seawater | 18800, 19040, 19470 | 18990, 18760, 18260 |

## 3.8 DETERMINATION OF CHROMIUM BY CYCLIC VOLTAMMETRY

Two different approaches were investigated for their potential to determine chromium(III) or chromium(VI), in the presence of the other oxidation state.

### 3.8.1 Determination of chromium(III)

#### 3.8.1.1 Instrumentation and conditions

The basic instrumentation configuration was as described elsewhere, section 3.2. The operating parameters were as described in section 3.2, except that for analysis the final potential was -1.5V, and the scan rate used was 400 mV $s^{-1}$.

Buffer control was achieved by addition of 100 µl of 1 M sodium acetate, and 100 µl of $1x10^{-2}$ M DTPA was added to complex the chromium. Aliquots of lead, from a stock of concentration $1x10^{-2}$ M were added to the solution to minimise the chromium(VI) signal.

#### 3.8.1.2 Lead concentration

Initial investigations were made into the effect of varying the concentration of the lead present in the cell. The effect of differing concentration of lead are summarised in table 22. The optimum concentration of lead based on these experiments was $5 \times 10^{-4}$ M. In all the subsequent experiments this was the concentration used.

It is worth noting that at this concentration of chromium(III), no interference from the lead was experienced in contrast to that noted previously, (section 3.6).

**Table 22 Effect of lead concentration on chromium(III) signal**

| Lead conc. ($x10^{-4}$ M) | Mean Peak height (mm) | Comments |
|---|---|---|
| 1 | 54 | Poor peak shape |
| 2 | 72 | |
| 5 | 84 | |
| 10 | 80 | Poor peak shape |

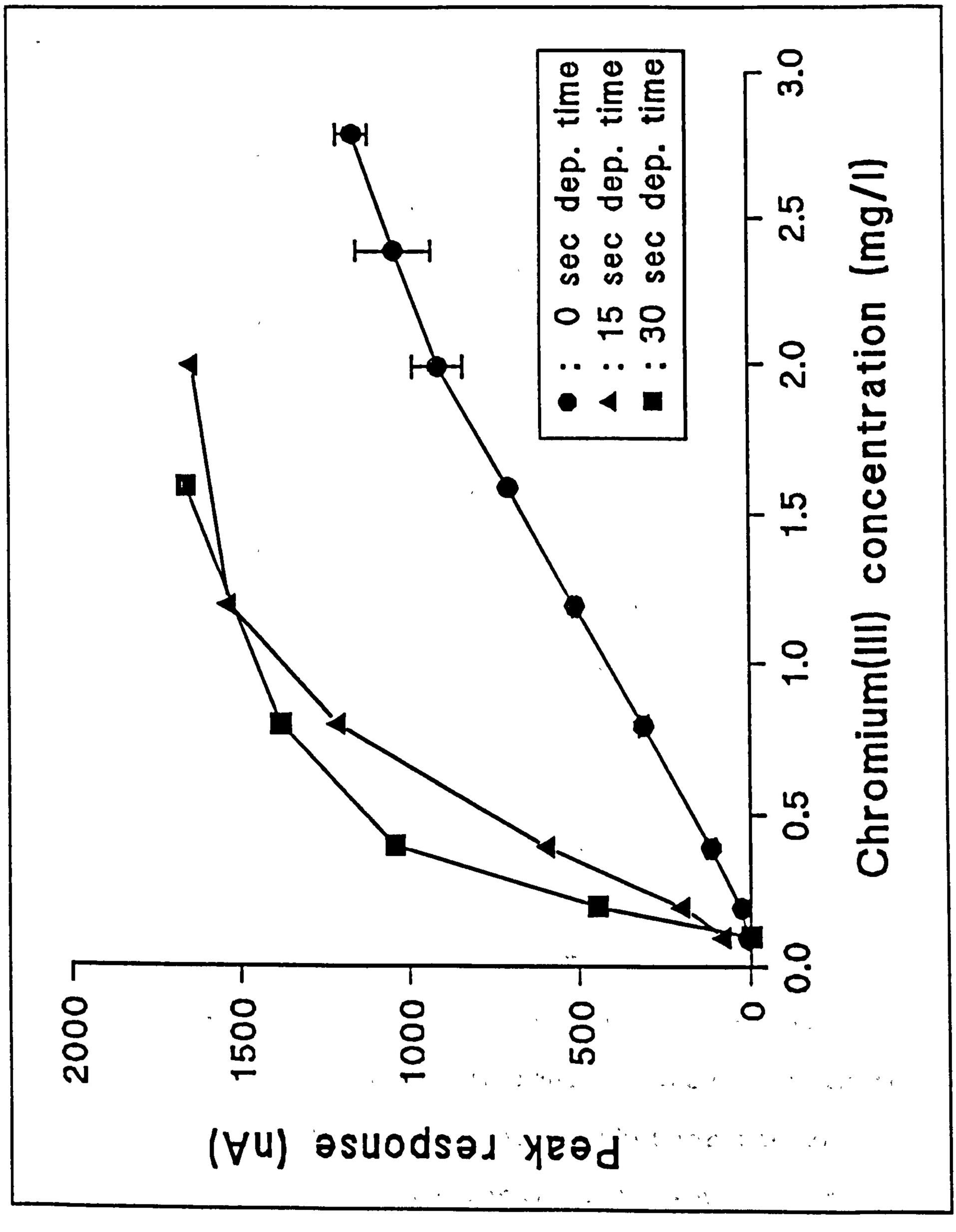
### 3.8.1.3 Deposition time

An investigation into the effect of the deposition time, figure 21, showed a linear response was only obtained when there was no deposition time. The most probable explanation of this finding was that the drop was saturated at this high concentration of chromium, rapidly leading to a plateau in the signal response. All subsequent experiments were carried out with zero deposition time.

### 3.8.1.4 Linearity, sensitivity and reproducibility

Using the method described above, further investigations of the electrochemical response were undertaken, in fresh and sea waters. The results of these experiments demonstrated that the method had acceptable analytical characteristics, and could be used in the determination of chromium(III) in waters, in support of aquatic toxicity tests. As expected the response of chromium(III) decreased with increasing salinity, however, as the

Figure 21 Effect of deposition time on chromium signal in the cyclic voltammetric analytical method



experiments were to be performed in model water, with known salinities it was decided that calibration for chromium(III) in 10, 20 and 30‰ seawater would be obtained when performing these toxicity experiments.

The effect of chromium(VI) on the chromium(III) signal was also evaluated by adding 1 to 3 $mgl^{-1}$ of chromium(VI) to the electrochemical cell. These investigations showed that 3 $mgl^{-1}$ chromium(VI) was equivalent to approximately 0.8 $mgl^{-1}$ chromium(III). This was higher than had been anticipated from earlier experiments, which had shown no interference at up to $1x10^{-4}$ M, (5.3 $mgl^{-1}$). A brief investigation showed that when lead was added to a solution already containing chromium(VI) no response was obtained, but that if the reverse were carried out, *i.e.* chromium(VI) was spiked into the cell, then a peak was obtained as described above. The probable explanation of this behaviour was that lead was complexed by excess DTPA if in the cell prior to the addition of chromium(VI). As the sample to be analysed would already contain chromium it was considered that there would be no interference from chromium(VI) under these conditions. However, this needs to be more fully investigated as to whether the above suggestion is correct. One possibility would be to investigate the effect of varying concentrations of DTPA on the chromium(VI) response, with a constant lead concentration. Thus other considerations apart, the signal due to chromium(VI) should increase with increasing DTPA signal, as lead is increasingly complexed by DTPA.

### 3.8.2 Determination of chromium(VI)

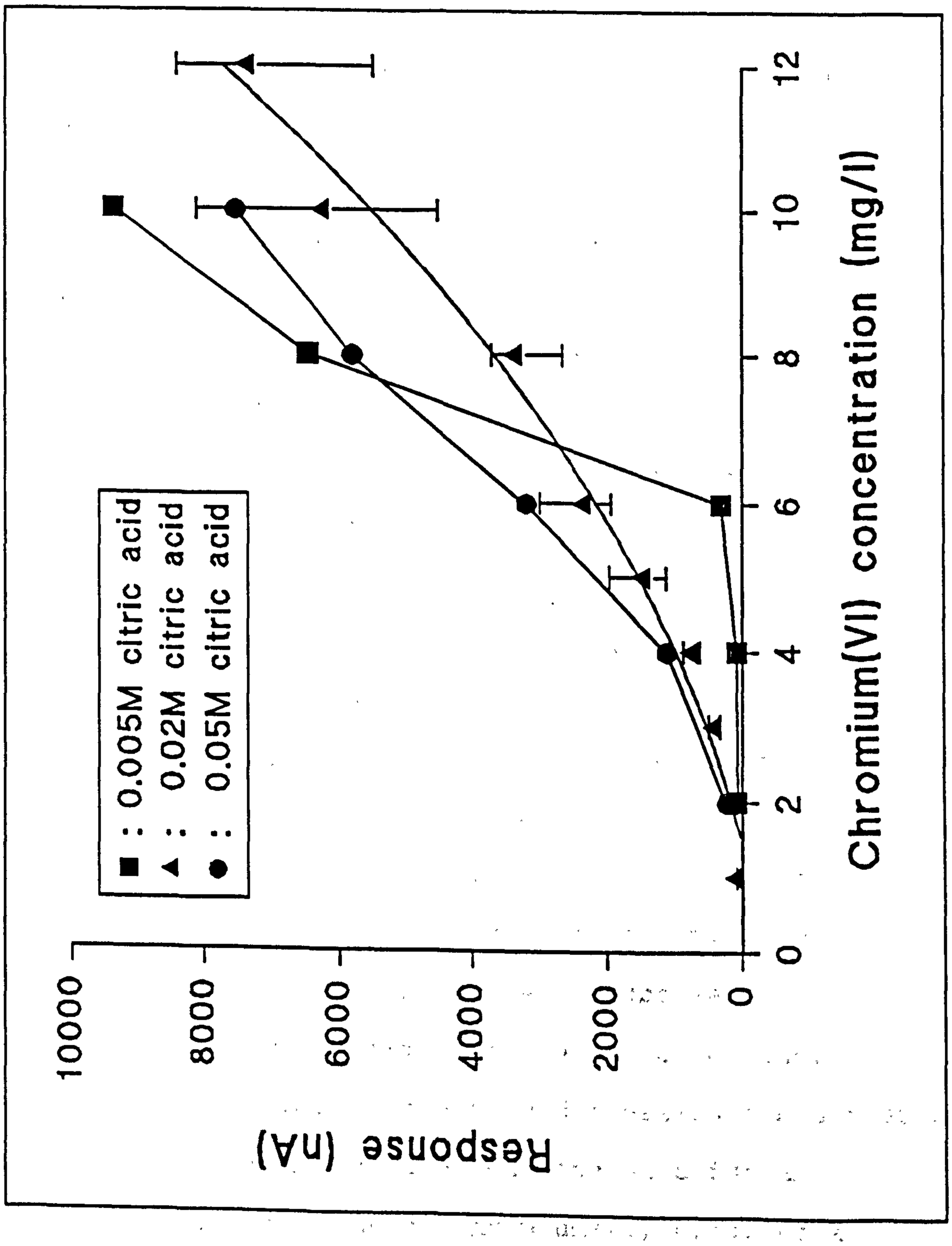
#### 3.8.2.1 Instrumentation and conditions

The basic instrumentation configuration was as described elsewhere, section 3.2. The operating parameters were as described in section 3.2, except that for these investigations the final potential was -1.5 V, and the scan rate used was 400 mV $s^{-1}$.

Buffer control was achieved by addition of 100 µl of 1 M sodium acetate, and 100 µl of $1x10^{-2}$ M DTPA was added to complex the chromium. Initially aliquots of citric acid, from a stock of concentration 2 M were added to the solution such that the final concentration of citric acid was 0.02 M in the cell. However, this resulted in a large drop in the pH, and subsequent experiments were carried out on prepared solutions of the reagents, pH adjusted to 6.2.

Under these conditions, the effect of increasing concentrations of chromium(VI) were investigated, figure 22. As may be seen in this figure the response at the lower concentration of citric acid suggested that there needed to be a minimum concentration of chromium(VI) present before a response was obtained. This was not noted at the higher concentrations of citric acid. This may possibly be because chromium(VI) was taking part in the catalysis of the response with citric acid. This is

Figure 22 Effect of citric acid concentration on the chromium(VI) signal in the cyclic voltammetric analytical method



supported by the sharp, tensammetric, shape of the chromium(VI) response at the higher concentrations of citric acid. At the lower concentration of citric acid, the chromium(VI) concentration needed to be above a minimum value, approximately 6 $mgl^{-1}$, before this reaction occurred.

There are two aspects of this response which require further investigation, the possible catalytic effect of citric acid, and the relationship between chromium(VI) concentration and that of citric acid, versus the response. The former may be better investigated by using the media exchange system described in section 3.9.

This method was not investigated further, as the response of chromium(VI) in seawater was minimal, and not quantifiable. This was most probably due to the complexation of citric acid in seawater, which thus led to a lower concentration of available citric acid ion. As noticed in figure 22 there needed to be a minimum chromium(VI) concentration before a response was obtained. Further work would confirm that the effect of increasing salinity would probably lead to a decreasing concentration of free citric acid ion, and thus the need for an increasing concentration of chromium(VI) prior to a response being obtained.

In view of the problems experienced with chromium(VI), the model solutions analysed in section 5, were analysed for

chromium(VI) using the technique described below in section 3.9, after appropriate dilution.

## 3.9 DETERMINATION OF CHROMIUM BY DPCSV WITH MEDIA EXCHANGE

The method and instrumentation used for the determination of chromium in an estuarine environment was as described below.

The configuration of the system is shown in figure 20.

### 3.9.1 Instrumentation

PAR 384B Polarograph, with a 303 HMDE and a Houston plotter,
Watson-Marlow peristaltic pumps, from Falmouth, Cornwall, Rheodyne low pressure switching valves, 6 and 4 way, from Anachem, Luton Beds.

### 3.9.2 Chemicals

Buffer solution 1 : 0.5 M DTPA, and 0.2 M sodium acetate, at pH 5.0;
Buffer solution 2 : 0.5 M sodium nitrate, and 0.02 M sodium acetate, at pH 5.0;

### 3.9.3 Methodology

At the start of this process the valves would be in

positions marked as follows:

Valve 1 - total (Cr6)

Valve 2 - total (Cr6)

Valve 3 - complexation (stripping)

the alternative positions are indicated in brackets.

1 With the valves set as described above, the sample was pumped through the cell for 10 minutes. Note only pump 1 was operating at this stage. In this position ammonia was flowing through the ion-exchange resin conditioning it for the determination of chromium(VI) later. The sample by-passed the resin and mixed with the complexation reagent prior to entering the electrochemical cell.

2 Pump 1 was then stopped, and the scan initiated by pressing RUN on the 384B. The sample was then degassed, and the chromium plated onto the mercury drop.

3 With 5s left of the equilibrium period PAUSE was pressed on the 384B, which then held the system in that position.

4 Valve 3 was then turned to the "stripping" position, and pumps 1 and 2 started. This exchanged the

solution in the electrochemical cell to that of the stripping reagent.

5 After 5 minutes, both pumps were stopped, and CONTINUE was pressed on the 384B. This gave the command to the electrochemical system to finish the equilibrium period, and then strip the chromium off the mercury drop, yielding the chromium response, which was recorded on the plotter.

6 When the scan was finished, valve 3 was returned to the "complexation" position, and valves 1 and 2 were turned to the "chromium 6" position. In this position the ammonia is diverted, and is not pumped, but the sample now goes through the resin column. Thus in this position no chromium(III) goes through to the electrochemical cell.

7 The cell was emptied, and the sample pumped through for about 2 minutes, prior to emptying the cell again. In this way the lines were cleared of the previous sample.

8 The procedure was then followed from step 1 again, but the sample was only be pumped for 5 minutes prior to step 2, as the previous sample had been cleared from the lines.

To standardise the system, standards of chromium(III) and

(VI) were used in place of samples. The standards were prepared in Milli-Q water, to obtain the maximum response from the system. Samples were quantified by standard addition in estuarine conditions, as the response for chromium(III) and (VI) depends on the salinity of the sample.

When operated in this mode it was possible to analyse a sample for total chromium and chromium(VI) approximately every 20 minutes.

# CHAPTER 4.....ENVIRONMENTAL MEASUREMENTS

## 4.1 INTRODUCTION

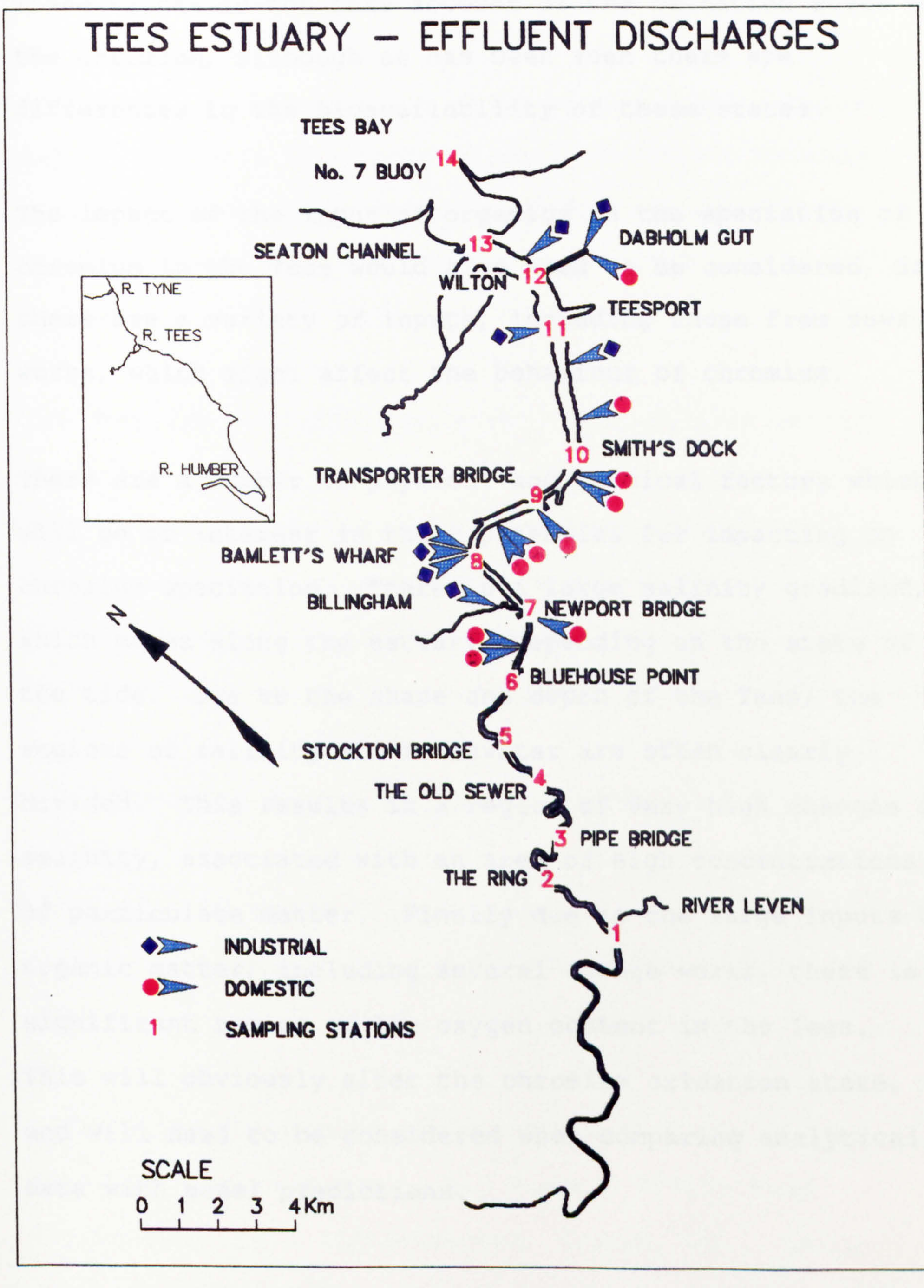
The area chosen for a monitoring exercise, was the Tees estuary. The Tees is one of the largest UK rivers discharging in to the North Sea, and is surrounded by a large concentration of industrial sites, mainly chemical, but also including steel works and a large oil refinery. The estuary is continuously dredged to maintain a channel deep enough to allow navigation by large ships.

Figure 23 shows the area of interest, with the major industrial sites, the principal sources of chromium and the chosen sampling sites.

As may be seen, the majority of the industrial sites are concentrated around the mouth of the estuary. However, there was one site thought to be a potential input of chromium higher up the river Tees, not shown in figure 1, above the confluence with the river Leven.

Although the Tees is considered to be grossly polluted, recently the discharges of sewage and industrial effluents have been controlled limiting the concentrations of discharged materials. In the case of chromium these controls are set to ensure the concentration does not exceed the Environmental Quality Standards, EQS, set by the UK government. At the time of writing the EQS in

**Figure 23 The River Tees - inputs and sampling positions**



force are 15 $\mu g l^{-1}$ in seawater, and 5 - 50 $\mu g l^{-1}$ in freshwater, based on average dissolved concentrations. These values do not take account of the oxidation state of the chromium, although as has been seen there are differences in the bioavailability of these states.

The impact of the input of organics on the speciation of chromium in the Tees would also need to be considered, as there are a variety of inputs, including those from sewage works, which might affect the behaviour of chromium.

There are a number of physical and chemical factors which will be of interest in their potential for impacting on chromium speciation. There is a large salinity gradient, which moves along the estuary, depending on the state of the tide. Due to the shape and depth of the Tees, the regions of salinity and freshwater are often clearly divided. This results in a region of very high changes in salinity, associated with an area of high concentrations of particulate matter. Finally due to the large inputs of organic matter, including several sewage works, there is a significant region of low oxygen content in the Tees. This will obviously alter the chromium oxidation state, and will need to be considered when comparing analytical data with model predictions.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Sampling positions

The sampling positions are shown in figure 23. These were mainly chosen on the basis of their proximity to known discharges. However, sampling points 1-3 and 14 were chosen to represent the input and the output of the estuary.

The sampling points in the main industrialised section of the estuary, 4-13, were also in the same region as the principal sampling areas used previously by ICI in sediment and water surveys of the Tees. By choosing these points it was intended that the data obtained for chromium in the waters, could be compared with known sediment data.

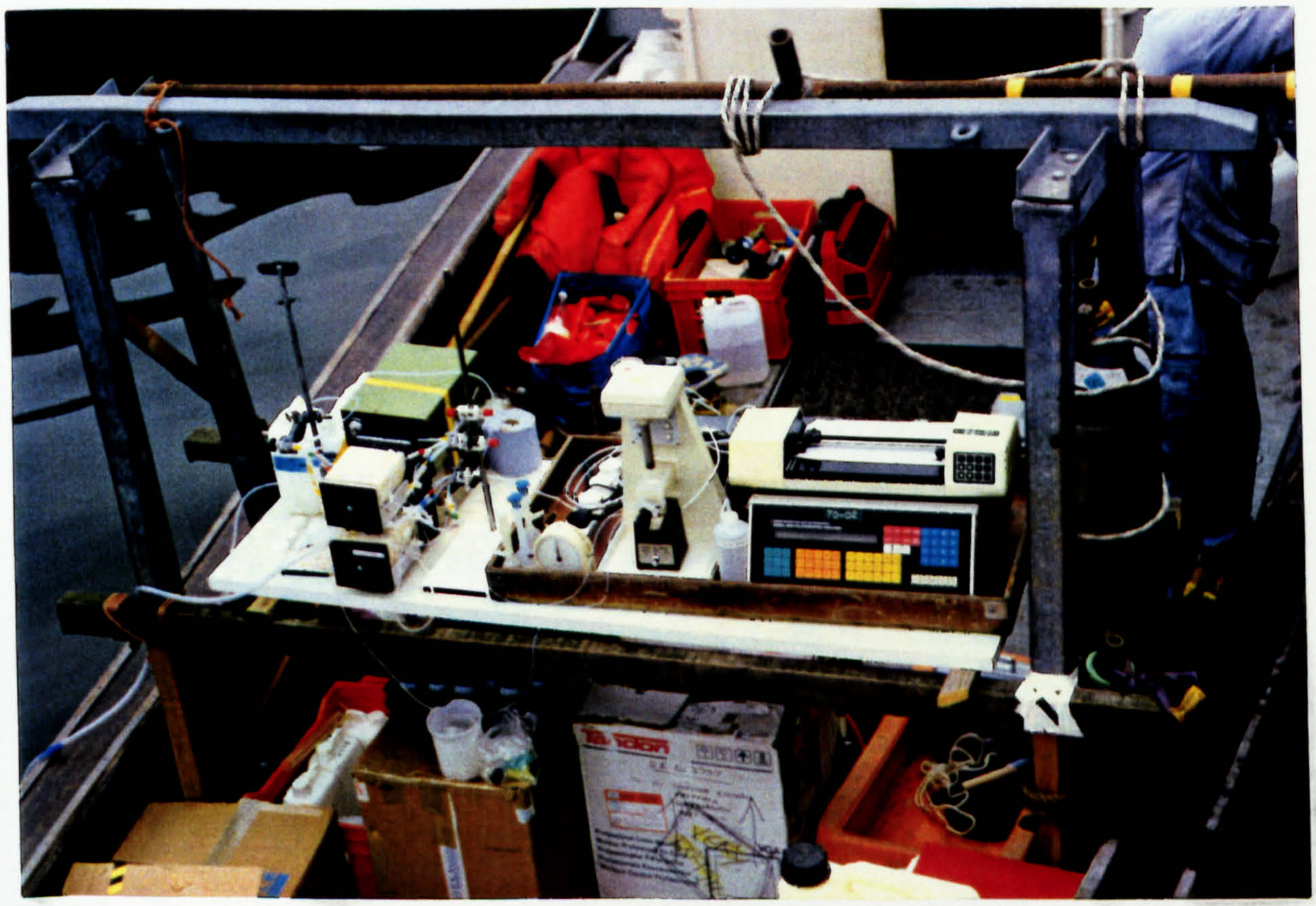
### 4.2.2 On-board sample collection system

The boat used was a 20 foot boat, with 4 foot draw, which enabled it to reach the confluence of the river Leven with the Tees.

River water was pumped up onto the boat using a gas operated diaphragm pump, (P&G Pageboy SFD15, Pump Engineering Ltd., Littlehampton, Sussex, UK). Using nitrogen supplied by a gas cylinder, obtained from BOC, (Whitby Rd, Bristol, UK), the pump was able to deliver a sample flow rate of approximately 1 litre $min^{-1}$. The

hosepipe line connecting the pump to the sample point was connected at the outlet to an interceptor vessel, where it was subsequently subsampled by the analytical system. Figure 24 shows the system as set up on board the boat. The incoming sampling point, was adjacent to a number of probes for the measurement of temperature, pH, salinity, and dissolved oxygen. Each of the probes had been calibrated in the laboratory, but the pH probe was also checked and recalibrated during the survey.

**Figure 24 On-board analytical system**



### 4.2.3 On-board procedures

The samples were collected following the procedure described below.

The sampling probe was lowered to 1 m, and the temperature, pH, salinity and dissolved oxygen of the water determined. Once these parameters had been determined a sample was taken from the stream of water, and the chromium determination procedure followed. When the electrochemical scan had been completed, the probe was lowered, to a depth just above the bottom of the river, and the process repeated.

At each point, as well as obtaining a sample for electro-chemical analysis, a sample was also taken in a PTFE bottle. These samples were later analysed for non-purgeable organic carbon and trace elements, including dissolved copper, nickel, iron, aluminium and chromium. The samples were also analysed for sodium, potassium, calcium and magnesium.

## 4.3 RESULTS

### 4.3.1 Physical-chemical measurements

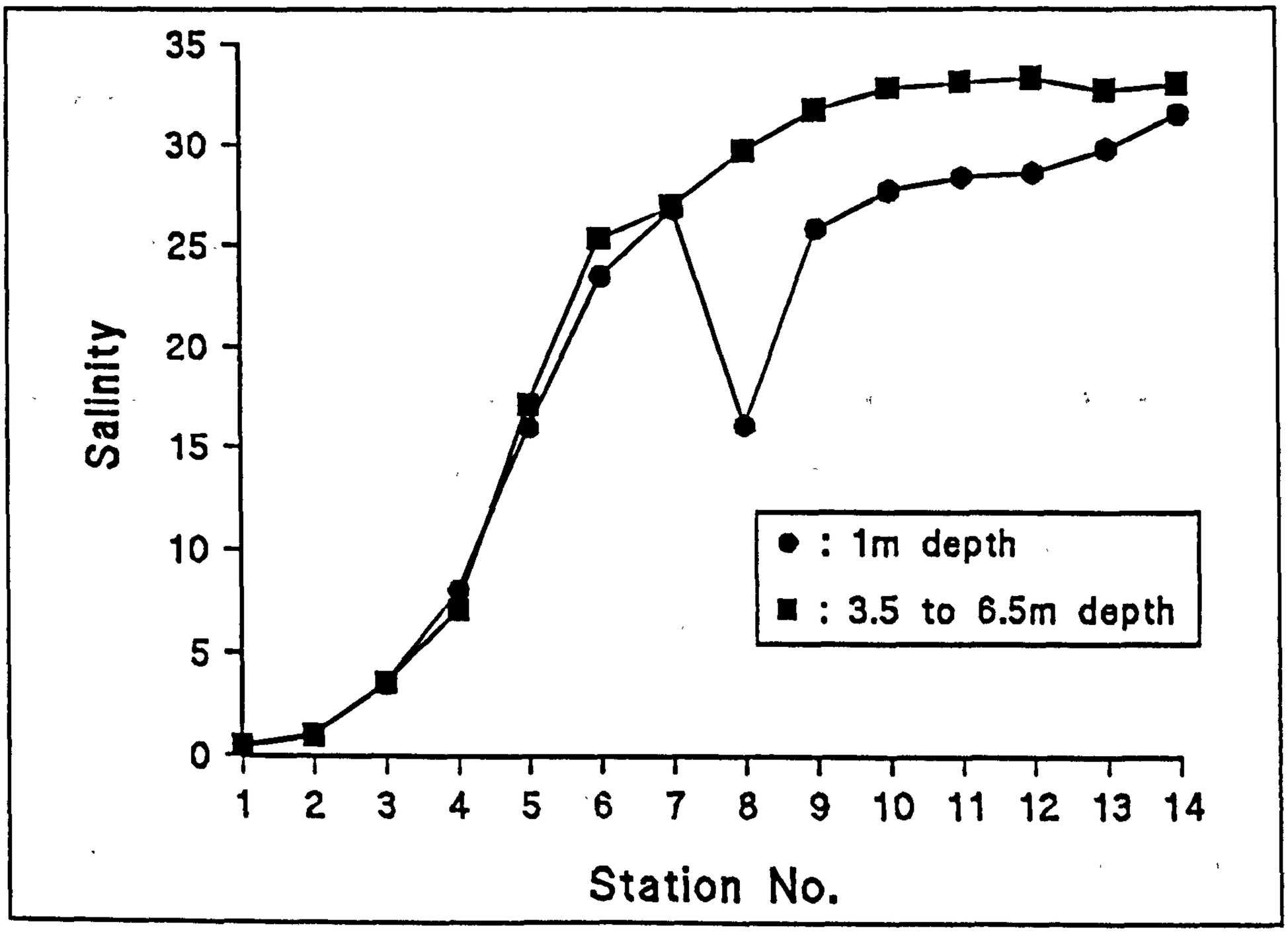
Table 23 presents the data obtained during the survey at the indicated sampling points. The times when the samples were obtained were recorded, high tide being at 10.17.

## Table 23 Sampling locations and physical-chemical results

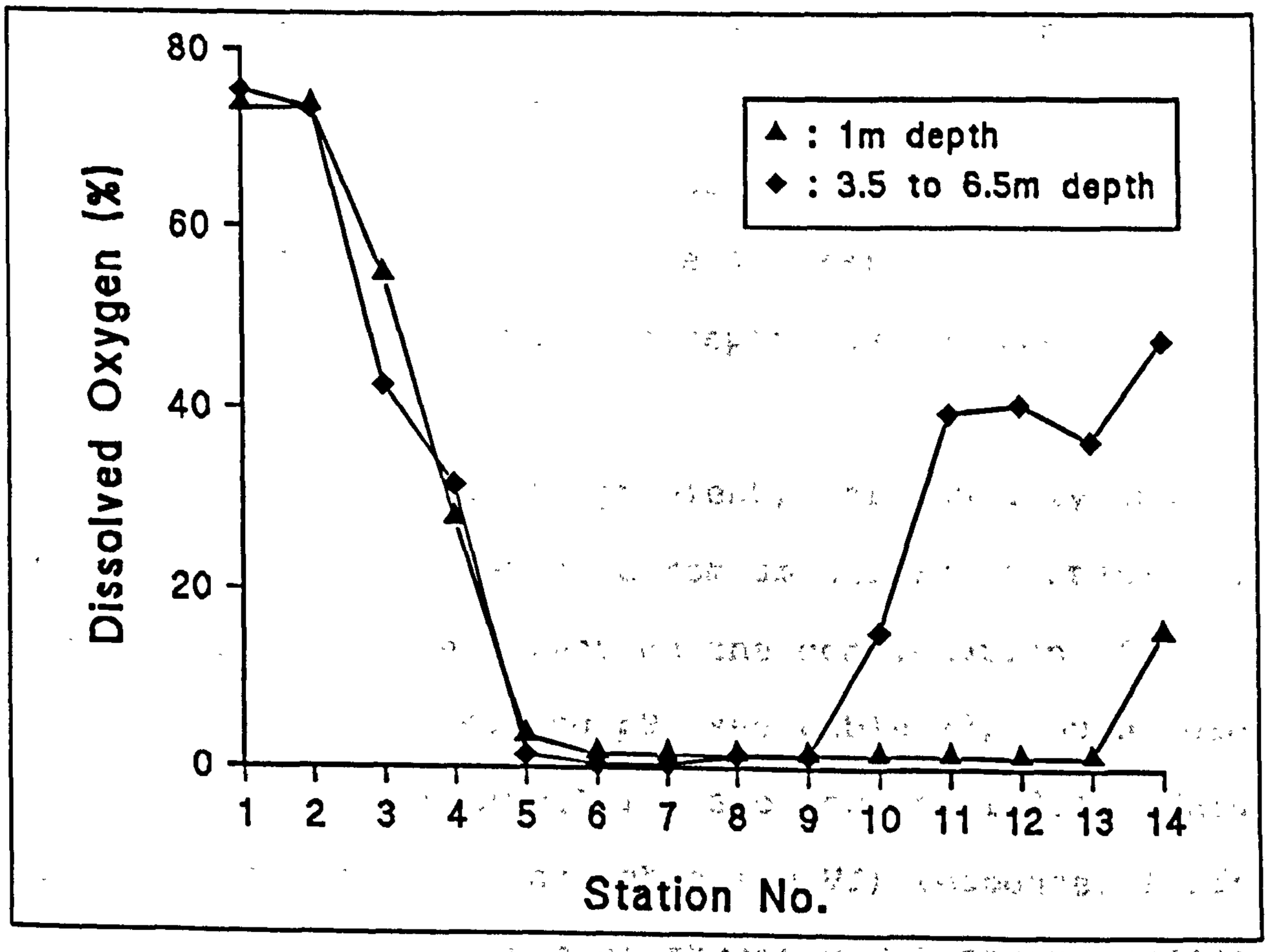
| Stat. # | Sampling Locations | Time | Depth (m) | pH | Sal. (‰) | Temp (°C) | DO (%) |
|---|---|---|---|---|---|---|---|
| 1 | River Leven | 11.05 | 1 | 5.9 | 0.5 | 14.4 | 74 |
| | | 11.21 | 3.5 | 5.5 | 0.5 | 14.35 | 76 |
| 2 | The Ring | 11.46 | 1 | 5.95 | 1.0 | 14.8 | 74 |
| | | 11.56 | 4 | 6.8 | 1.0 | 14.8 | 74 |
| 3 | Pipe Bridge | 12.16 | 1 | 5.4 | 3.5 | 15 | 55 |
| | | 12.27 | 4 | 6.6 | 3.6 | 14.9 | 43 |
| 4 | The Old Sewer | 12.43 | 1 | 7.0 | 8.0 | 14.9 | 28 |
| | | 12.54 | 4 | 6.6 | 7.1 | 14.8 | 32 |
| 5 | Victoria Bridge | 13.11 | 1 | 6.9 | 16 | 14.4 | 4 |
| | | | 4 | 6.7 | 17.2 | 14.2 | 2 |
| 6 | Blue House Pt | 13.32 | 1 | 6.8 | 23.5 | 14 | 2 |
| | | 13.35 | 4 | 6.65 | 25.4 | 13.45 | 1 |
| 7 | Newport Bridge | 13.46 | 1 | 6.8 | 26.75 | 13.45 | 2 |
| | | 13.51 | 4 | 6.4 | 27 | 13.4 | 1 |
| 8 | Bamlett's Wharf | 17.15 | 1 | 6.6 | 16.1 | 14.8 | 2 |
| | | 17.17 | 4 | 6.1 | 29.8 | 13.05 | 2 |
| 9 | Transporter's Bridge | 17.00 | 1 | 7.2 | 25.8 | 13.95 | 2 |
| | | 17.05 | 6.5 | 6.7 | 31.8 | 12.8 | 1.5 |
| 10 | Smith's Dock | 16.30 | 1 | 7.2 | 27.7 | 13.9 | 2 |
| | | 16.35 | 6.5 | 6.8 | 32.9 | 12.6 | 16 |
| 11 | Tees Dock | 16.13 | 1 | 7.3 | 28.4 | 13.9 | 2 |
| | | 16.16 | 6 | 7.4 | 33.2 | 12.8 | 40 |
| 12 | Wilton | 14.47 | Surf. | 7.2 | 28.6 | 14 | 5 |
| | | 14.49 | 1 | 7.2 | 28.6 | 13.45 | 2 |
| | | 14.56 | 6.5 | 7.2 | 33.35 | 12.6 | 41 |
| 13 | Seaton Channel | 15.50 | 1 | 7.4 | 29.75 | 13.45 | 2 |
| | | 15.53 | 6.5 | 7.45 | 32.75 | 13.0 | 37 |
| 14 | No 7 Buoy | 15.35 | 1 | 7.35 | 31.5 | 13.2 | 16 |
| | | 15.37 | 3.5 | 7.4 | 33.12 | 12.8 | 48 |

Figure 25 Dissolved oxygen and salinity results

a) Salinity




b)Dissolved oxygen



The dissolved oxygen and salinity data are plotted in figure 25. The most noticeable features of these curves are the drop in dissolved oxygen levels and the probable presence of the salinity wedge at station 8.

#### 4.3.1.1 Salinity profile - Tees estuary

At the start of the survey, the tide had turned, high tide at the mouth of the estuary being 10.17. At the early stations although there was some evidence of salt water, the data obtained suggested that the river was at this point well mixed. A salinity gradient did not begin to appear until station 5. This was not unexpected, as the period of maximum mixing would be when the fresh and sea water zones were moving in opposite directions.

As the survey progressed, and the amount of mixing decreased, low water at the mouth of the estuary being 16.49, then the two zones would be more obvious. This may be seen at stations 8 - 14, where a clear difference between the salinity at the two depths is apparent.

The effect of this salinity gradient, horizontally and vertically, on the different chromium oxidation states is unclear. However, the effect on the complexation of chromium(III), of increasing pH, see table 23, would lead to less available chromium(III), see section 1.2.1. This in turn, assuming a constant chromium(VI) response, leads to an increase in the ratio of chromium(VI):chromium(III).

#### 4.3.1.2 Dissolved oxygen

There was a rapid decline in the level of dissolved oxygen starting at station 3, and culminating in less than 1% by station 5. This is up river from the major effluent discharges, but is consistent with it being high tide, with the water being pushed up river. This would most probably lead to a decrease in the chromium(VI) : chromium(III) ratio, as chromium(VI) would be reduced in such anoxic conditions, and/or chromium(III) would either be produced, from chromium(VI) reduction, or less chromium(III) would be oxidised to chromium(VI).

The other area of interest was the seaward end of the estuary where there was some stratification, noticed in section 4.3.1.1 for the salinity. Thus from station 10 downwards, there is a difference between the lower oxygenated seawater, and the overlying anoxic freshwater.

This should also lead to differences in the ratio of chromium(VI) : chromium(III), in that in the lower, oxygenated level, there will be a high ratio of chromium(VI) : chromium(III), while in the freshwater, the ratio will be considerably lower.

In summary then, the data obtained for oxygen and salinity in the estuary, leads to some interesting speculation as to the likely change in chromium speciation, and its impact on the chromium(VI) : chromium(III) ratio. The following section, will describe the attempt to measure

chromium, and hence determine if the discussions above are valid. These discussions have, however, not taken into account the possibility of sources of chromium of differing speciation.

### 4.3.2 Chromium

Initial results were encouraging and indicated that chromium was present, but at very low levels. Thus at station 1, concentrations of 0.3 and 0.2 $\mu g l^{-1}$ were obtained for chromium(VI) at 1 m and 3.5 m depth respectively. At station 2, the levels measured were lower being 0.2 and <0.1 $\mu g l^{-1}$, again as chromium(VI), at 1 m and 4 m respectively. However, these values were based on comparison with the de-ionised water standards run prior to the river water samples. This was considered acceptable, as at these points the salinity was 0.5 and 1‰ respectively.

However, as the salinity had increased at the next sampling point, it was decided to quantify the chromium present by standard addition. At this point it was discovered that the system was not responding with the expected sensitivity. Although this could have been a natural response to organic complexation in the samples, attempts to recalibrate the electro-chemical system with de-ionised water, demonstrated that the response to chromium had been lost.

By systematically changing the working electrode, and then the reference electrode, it was discovered that the problem was due to the reference electrode. With a new reference electrode a normal response for chromium(VI) and (III) was obtained in de-ionised water. However, when river water was introduced into the system the response was again lost. Investigations during the course of the survey suggested that this effect was irreversible. Thus passing de-ionised water through the cell, and attempting to obtain a chromium response were unsuccessful, although water was pumped for over an hour.

Visual examination of the frit, made of KelF wax, did not show any obvious contamination. Furthermore, immersing the frit in 0.02 M ammonia or 0.02 M hydrochloric acid was not successful in removing the interference.

As it was no longer possible to obtain data for chromium concentrations in the field, it was decided to take extra samples for analysis for chromium in the laboratory. As the stability of chromium(III) and (VI) is suspect, although it had been stated to be promising over short periods in PTFE, (102), it was decided that some samples would be spiked with chromium(III) and (VI), in an attempt to validate the proposed storage of these samples. A sample of de-ionised water was also spiked with chromium(III) and (VI).

In the laboratory, little further information was

obtained. An attempt to quantify the interference was made by adding aliquots of the river water to a de-ionised water system containing 5 μg $l^{-1}$ of chromium(VI). This showed that in the sample chosen, station 4, at least 10% river water was necessary, before a discernible effect on the peak height was noticed. However, to be fully useful, this experiment needed to be performed on all the samples, thus building a profile of the interference in the river water samples. This would have consumed a large number of reference frits, and in view of the amount of time needed to do this, and that the stability of the response could not be confirmed, it was decide not to do this.

However, the result from station 4 may be compared with 50% at station 1 and 20% at station 14. This suggests that the interference may be reasonably constant within the main body of the estuary, and is almost certainly introduced somewhere in the upper estuarine region. This is a phenomenon which should be further investigated.

### 4.3.3 Elemental results

Table 24 summarises the results obtained from Inductively Coupled Plasma Optical Emission Spectrometry, ICP-OES, analysis of the samples for a range of elements including chromium. As noted in the footnote to this table, many of the trace metal results are within the area of uncertainty for the accurate determination of these elements,

**Table 24 Summary of analytical measurements (see table 23 for station locations)**

| Stat # | Depth (m) | Fe | Al | Ni | Cr | NPOC* | Na | K | Ca | Mg |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (µg/l) | | | | (mg/l) | | | | |
| 1 | 1 | 18 | <150 | <20 | <10 | 3.9 | 241 | 15 | 59 | 39 |
| | 3.5 | 26 | <150 | <20 | 18 | 4.6 | 231 | <5 | 70 | 39 |
| 2 | 1 | 19 | <150 | <20 | 12 | 6.1 | 429 | 18 | 77 | 58 |
| | 4 | 11 | 393 | <20 | 12 | 7.5 | 209 | <5 | 70 | 32 |
| 3 | 1 | 22 | 372 | <20 | 12 | 5.6 | 1213 | 29 | 103 | 139 |
| | 4 | 26 | <150 | <20 | <10 | 4.5 | 1668 | 33 | 117 | 190 |
| 4 | 1 | 27 | <150 | 73 | 18 | 5.0 | 3080 | 93 | 163 | 346 |
| | 4 | 30 | 382 | <20 | 11 | 5.0 | 2754 | 71 | 153 | 306 |
| 5 | 1 | 49 | 402 | <20 | 13 | 4.5 | 5784 | 183 | 247 | 648 |
| | 4 | 53 | 254 | <20 | 11 | 4.3 | 6455 | 208 | 274 | 735 |
| 6 | 1 | 50 | <150 | 23 | 72 | 15.0 | 6347 | 214 | 269 | 703 |
| | 4 | 72 | <150 | 23 | 18 | 8.0 | 9340 | 300 | 370 | 1050 |
| 7 | 1 | 54 | 415 | <20 | 20 | 3.5 | 9526 | 305 | 378 | 1102 |
| | 4 | 62 | 411 | <20 | 18 | 10.0 | 9861 | 308 | 383 | 1121 |
| 8 | 1 | 192 | <150 | <20 | <10 | 8.0 | 5907 | 201 | 259 | 684 |
| | 4 | 63 | <150 | <20 | <10 | 4.2 | 5907 | 201 | 259 | 684 |
| 9 | 1 | 24 | <150 | <20 | <10 | 2.4 | 10186 | 323 | 406 | 1175 |
| | 6.5 | 95 | <150 | <20 | <10 | 2.7 | 9136 | 306 | 362 | 1046 |
| 10 | 1 | 12 | <150 | 69 | 23 | 3.0 | 11291 | 364 | 437 | 1300 |
| | 6.5 | <10 | <150 | <20 | <10 | <1.0 | 10683 | 354 | 411 | 1226 |
| 11 | 1 | 38 | <150 | 72 | 24 | 4.8 | 10989 | 342 | 415 | 1268 |
| | 6 | 20 | <150 | 48 | 26 | 4.8 | 11166 | 336 | 430 | 1312 |
| 12 | Surf | 43 | <150 | 61 | 21 | 4.6 | 10252 | 331 | 404 | 1169 |
| | 1 | 48 | <150 | 49 | 26 | 3.2 | 9964 | 311 | 391 | 1162 |
| | 6.5 | 11 | <150 | 49 | 20 | <1.0 | 11259 | 358 | 423 | 1284 |
| 13 | 1 | 37 | <150 | 87 | 21 | 9.6 | 10843 | 351 | 413 | 1249 |
| | 6.5 | 26 | <150 | 51 | 27 | 5.6 | 9834 | 306 | 388 | 1158 |
| 14 | 1 | 16 | <150 | 62 | 23 | 5.6 | 10699 | 347 | 409 | 1238 |
| | 3.5 | <10 | <150 | 45 | 25 | | 10752 | 354 | 419 | 1273 |

**NB : The data above, notably that for the trace metals, iron, aluminium, nickel and chromium must be treated with caution, and should be used as indicators of a trend, not as definitive levels to be found in the Tees.**

* NPOC : Non-purgeable organic carbon

approximately 3 x the limit of detection. For this reason the data should be treated with caution, and only general trends may be examined.

#### 4.3.3.1 Major elements

As might be expected, the major seawater cations determined, sodium, potassium, magnesium and calcium, all show a similar trend of concentration versus station number, to that of salinity, see table 23. It is possible to compare the data obtained during this study, with that predicted when setting up the model, table 5 section 2.3.

Thus at station 5, at 1 m depth, a salinity of 15‰ was measured. In the corresponding sample 251 mM sodium and 27 mM magnesium was determined. These values compare with 208 mM sodium and 24 mM magnesium obtained in the model. Similarly at station 14, also at 1 m depth, the measured values for salinity, sodium and magnesium were 33.12‰, 465 and 52 mM. While in the model, the corresponding sodium and magnesium concentrations at 30 and 35‰ salinity, were 416 and 485, and 47.2 and 55 mM.

The measured data do, therefore, follow the general trend expected, and used in the model, of conservative mixing between the river and seawaters.

### 4.3.3.2 Trace metal results

a) Aluminium

The aluminium results show, that apart from a few samples in the upper estuary, the level of aluminium was below the limit of detection of the method used, 150 μg $l^{-1}$, 5 mM. However, both the occasional results obtained, and the detection limit are in excess of the results expected in the model, table 5, section 2.3, ranging from 0.19 to 0.08 mM.

The impact of aluminium on chromium speciation has not been explored, but it is improbable that the hydroxidation of aluminium at these levels would have much impact on chromium. However, it is suggested that future surveys should consider the possibility, and a more sensitive method for the determination of aluminium used.

b) Nickel

The concentrations of nickel found were much higher than were being used in the model, at approximately 800 nM, compared with a suggested value of around 30 nM, table 5, section 2.3. It is also clear from the data in table 24 that there is a discharge near the mouth of the estuary, thus leading to the elevated levels noted. For the purposes of this exercise, however, the concentration of nickel, even at these levels will probably not effect the speciation of

chromium.

c) Copper

The detection limits of the analytical method used are relatively high for determining environmental levels of copper. However, there have been occasions in the past when such a method would have been sufficient to determine levels of dissolved soluble copper in the region of stations 6 - 8, and 11 - 13, (93, 103).

d) Iron

The data obtained in this study for dissolved total iron, *i.e.* no attempt was made to measure the seperate oxidation states, suggest that the levels of iron were reasonably constant in the Tees estuary, with over 70% of the results less than 50 $\mu g^{-1}$, *i.e.* less than 900 nM. The data used in the model was based on the assumption of conservative mixing and using a seawater concentration of 1900 nM, table 5 section 2.3. It is possible that iron is not conservatively mixed, but precipitates or adsorbs to the suspended material along the estuary. This was investigated by Salomons, (104), who found that iron was precipitated at high salinity and pH.

### 4.3.4 Organic carbon

Table 24 shows the results obtained from the determination

of the dissolved organic carbon in the samples. All these data are after filtration of the samples through a 0.45 μ glass fibre filter. Unfortunately this step introduced a very large carbon blank, and these data must be treated with extreme caution.

The data do suggest, however, that the inputs in the middle reaches of the estuary, a region of industrial and domestic discharges, do have an effect on the levels in the estuary. However, an improved detection limit, and by filtering the samples through a better filter, *e.g.* membrane filters, a better pre-treatment process is needed, together with a method of characterising the organic matter. In this way it would be possible to comment on the impact of the organic matter in the Tees on the speciation of chromium.

One point worth noticing, is that if a molecular weight of 500 is assumed for the organic matter, then 10 mg $l^{-1}$ of carbon, corresponds to at least 0.02 M of organic matter. This compares with a range in table 5, see section 2.3, of 0.003 to 0.03 mM.

## 4.4 SUMMARY

Although it was not possible to measure chromium(III) and (VI) concentrations in the Tees, and thus compare the results with predictions made by the model, a number of

findings were made that should be further investigated.

The major discovery was of an unknown interference, which appeared to adsorb irreversibly to the frit of the reference electrode within the electro-chemical system being used in this study. The source and identity of this interference should be investigated. The most obvious way the source could be found would be by reproducing on the estuary, the experiment described above in section 4.3.2. Thus as the source, or possibly sources, was neared the amount of river water necessary to poison the reference would diminish. The major problem with this approach is the potentially large number of frits which would be consumed in the process. Clearly an alternative method, either with a more resistant frit, or perhaps through coating the frit, and measuring the current across such a membrane, needs to be investigated.

The possible use of an external reference electrode was briefly investigated, however, there was insufficient time to evaluate properly whether such an approach would have proven useful in this instance. This is an experiment that should be more fully investigated.

The importance of measuring a range of parameters was emphasised by the measurement of the oxygen depletion in the middle estaury. Further the salinity and pH measurements were also seen to be important as variations in metal speciation could be related to these parameters,

section 4.3.1.

Finally it was demonstrated that future surveys of the speciation of metals, should ensure that other metals, *e.g.* nickel, iron and aluminium are accurately measured. It is also necessary to assess the levels and type of organic matter present in the water body being surveyed, if the behaviour of the metal being investigated is to be more fully understood.

# CHAPTER 5.....AQUATIC TOXICITY EXPERIMENTS

It should be acknowledged that three other people carried out the practical biological work reported in this chapter. Stuart Beckhurst, who conducted the brown shrimp tests, *Crangon crangon*, Tom Hutchinson who carried out the Sheepshead Minnow tests, *Cyprinodon variegatus*, and Tim Williams, who was responsible for the *Tisbe* tests, *Tisbe batagliai*. I am grateful to them, for their contribution to this programme and for their comments on the data within this thesis.

## 5.1 INTRODUCTION

The data in Chapter 1, showed that although there was a considerable body of data relating to the toxicity of chromium to aquatic organisms, little of it was applicable to estuarine species, or strictly capable of comparing the effect of the two oxidation states. There have also been few reported studies looking at the impact of chromium interactions with organics, and how this impacts on chromium aquatic toxicity.

Initial experiments were aimed at providing comparable data for chromium(III) and (VI), while investigating the impact of estuarine conditions, and what differences were apparent in the impact of the oxidation states with different types of animal. The species chosen for these experiments were the brown shrimp, *Crangon crangon*, tested

at 20 and 30 ‰ salinity, and the sheepshead minnow, *Cyprinodon variegatus*, which was tested in seawater.

It was also intended to investigate the effect of chromium at levels closer to those found in the environment, and the impact of organic chelators. For these experiments the organism chosen was the copepod, *Tisbe batagliai*. The principal reason for choosing this organism was its sensitivity, but there are other advantages to be discussed in more detail later, including sample size and the relatively short timescale of the test.

## 5.2 TOXICITY OF CHROMIUM(III) AND (VI) TO *Crangon crangon*, IN ESTUARINE CONDITIONS

The procedure used was a recognised method for the testing of the effect of chemicals to the brown shrimp, and at the time the studies were carried out, the protocol used was based on that used to support registration of chemicals for use in the North Sea. The test was a 96 hour semi-static study, with renewal of the test solutions every 24 hours.

The brown shrimp has been used regularly in tests of this nature as it is easy to obtain, and relatively easy to handle. Although considered a relatively insensitive species, it is nevertheless indicative of the type of animal found in the lower reaches of an estuary. With varying salinities, and oxygen levels they must be hardy

organisms, and so are naturally resistant to a range of otherwise harmful materials.

### 5.2.1 Materials and methods

#### 5.2.1.1 Chemicals and test solutions

The two chromium salts used, chromic chloride and potassium dichromate, were obtained from BDH Poole, Dorset. Exposure solutions were made up by making a stock solution in seawater, and diluting appropriate volumes of these stocks in 20‰ and 30‰ seawater.

#### 5.2.1.2 Toxicity test method

The glass test vessels were of 10 litre capacity, and at each test concentration, 20 animals were tested. During the study the test solutions were gently aerated with air passed through a pasteur pipette. The test vessels were held at 15±1°C, and a time clock was used to control the photoperiod to 16 hours of light, followed by 8 hours of darkness.

The test was started by placing 20 animals in each vessel, and recording mortalities at 1, 24, 48, 72 and 96 hours.

In some instances, animals moult during the test, and are subsequently cannibalised. Such moult deaths, although recorded are not included in the calculations of mortality

for the test populations.

From the recorded deaths at 24, 48, 72 and 96 hours, the LC50, (median lethal concentration), at those times, and their confidence intervals were calculated using the method of Stephan, (105).

### 5.2.1.3 Physical and chemical analyses

The pH, dissolved oxygen, salinity and temperature values were measured in each of the vessels, before and after the test solutions were renewed.

Model solutions of the two chromium salts, at the respective nominal concentrations were also analyzed, using the procedure described in sections 3.8 and 3.9. Total chromium was also measured using direct nebulisation into an ICP-OES instrument.

## 5.2.2 Results

The mortality data for chromium(III) and (VI) are summarised in Tables 25 and 26, respectively.

The physical parameters of the dilution water used each day had a pH range of 8.24 - 8.32, hardness (as $CaCO_3$) of 61.6 - 63.3 mg $l^{-1}$, an alkalinity (as $CaCO_3$) of 28.2 - 30.2 mg $l^{-1}$ and a salinity (‰) of 34.96 - 35.05.

The data from the chemical analyses are summarised in tables 27 and 28.

The derived LC50 values are presented in table 29 for all 4 studies, and shown in figure 26.

### 5.2.3 Discussion

Analysis of the chromium(III) model solutions showed that little chromium(VI) was formed. However, this data was only useful for a qualitative demonstration of the lack of conversion of chromium(III) to (VI). The dilution factors involved meant that there was considerable potential for errors to occur in generating quantitative data. The results obtained did show that the chromium(III) concentration did decline with time, and a precipitate was observed at the higher concentrations.

Analysis of chromium(VI) model solutions was also very difficult, involving serial dilutions in excess of 1000. The data did confirm that chromium(VI) remained in solution. The problem experienced with chromium(VI) interferring with the determination of chromium(III), discussed in section 3.8, meant that at many of the higher concentrations of chromium(VI) it was not possible to determine if any chromium(III) was formed. These are described as ND, not determined, in table 28. The data for the lower levels suggests that there was practically no conversion of chromium(VI) to (III), and that the

toxicity noted in these solutions was due to chromium(VI),

Investigation of the speciation of chromium(III) at these concentrations in 20 and 30‰ salinity, suggested that the major form is $Cr(OH)_4^-$. This is a relatively insoluble salt, and there was some loss of chromium from both sets of model solutions with time. This precipitation could by itself have lead to a toxic effect by, for example, clogging of the gill surfaces of the animals. However, the shape of the measured and nominal curves are similar, suggesting that it is the dissolved chromium(III) which is causing the effect. This is apparent at both salinities.

The effect of chromium(VI) at both salinities shows two interesting properties. In both cases early in the tests chromium(VI) appears to be considerably less toxic than chromium(III). However, as the time interval increases then the difference between the two oxidation states lessens, and by 96 hours, at 30‰ salinity chromium(VI) is more toxic than chromium(III). The second feature of both chromium(VI) curves is the way they appear to indicate that with increasing time, chromium(VI) will become increasingly more toxic. One interpretation of this data, could be that chromium(VI) is moving into the animal's body, exerting a toxic effect within the organism, in such a way that the effect requires time before being expressed as a toxic response. Chromium(III), however, appears to be having a more immediate effect, and is not accumulating in the animal's body.

**Table 25 Cumulative percentage mortality of *Crangon crangon* exposed to chromium(III) in 20‰ and 30‰ salinity**

a) 20‰ salinity (nominal concentrations)

| Conc ($mgl^{-1}$) | % Cumulative Mortality | | | | |
|---|---|---|---|---|---|
| | 1 hr | 24 hr | 48 hr | 72 hr | 96 hr |
| Control | 0 | 0 | 0 | 0 | 0 |
| 3.5 | 0 | 0 | 0 | 0 | 0 |
| 6.2 | 0 | 0 | 0 | 0 | 6 |
| 11 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 5 | 6 |
| 35 | 0 | 5 | 5 | 42 | 74 |
| 62 | 0 | 40 | 90 | 100 | 100 |

b) 30‰ salinity (nominal concentrations)

| Conc ($mgl^{-1}$) | % Cumulative Mortality | | | | |
|---|---|---|---|---|---|
| | 1 hr | 24 hr | 48 hr | 72 hr | 96 hr |
| Control | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 |
| 35 | 0 | 5 | 11 | 11 | 11 |
| 62 | 0 | 10 | 21 | 53 | 72 |
| 109 | 0 | 16 | 63 | 95 | 100 |

**Table 26 Cumulative percentage mortality of *Crangon crangon* exposed to chromium(VI) in 20‰ and 30‰ salinity**

a) 20‰ salinity (nominal concentrations)

| Conc ($mgl^{-1}$) | % Cumulative Mortality | | | | |
|---|---|---|---|---|---|
| | 1 hr | 24 hr | 48 hr | 72 hr | 96 hr |
| Control | 0 | 0 | 0 | 0 | 0 |
| 2.7 | 0 | 0 | 0 | 0 | 6 |
| 4.8 | 0 | 0 | 0 | 0 | 6 |
| 8.6 | 0 | 0 | 0 | 6 | 7 |
| 15 | 0 | 0 | 0 | 6 | 17 |
| 27 | 0 | 0 | 0 | 0 | 16 |
| 48 | 0 | 0 | 11 | 32 | 74 |
| 86 | 0 | 0 | 25 | 75 | 90 |
| 150 | 0 | 30 | 80 | 100 | 100 |

b) 30‰ salinity (nominal concentrations)

| Conc ($mgl^{-1}$) | % Cumulative Mortality | | | | |
|---|---|---|---|---|---|
| | 1 hr | 24 hr | 48 hr | 72 hr | 96 hr |
| Control | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 |
| 27 | 0 | 0 | 0 | 0 | 53 |
| 48 | 0 | 0 | 5 | 41 | 65 |
| 86 | 0 | 5 | 35 | 89 | 100 |
| 150 | 0 | 25 | 90 | 100 | 100 |
| 270 | 10 | 100 | 100 | 100 | 100 |

**Table 27 Chemical analyses of model solutions containing chromium(III) in 20% and 30% salinity**

NB : The chromium(VI) data must be treated with caution, as the samples were diluted x100 prior to analysis.

| Conc ($mgl^{-1}$) | Measured Concentration (mg/l) | | | |
|---|---|---|---|---|
| | 0 hr | | 24 hr | |
| | Cr(III) | Cr(VI) | Cr(III) | Cr(VI) |
| 20% Salinity | | | | |
| Control | <0.5 | <0.1 | <0.5 | <0.1 |
| 3.5 | 3.4 | <0.1 | 3.5 | <0.1 |
| 6.2 | 6 | <0.1 | 6 | <0.1 |
| 11 | 10 | <0.1 | 10 | <0.1 |
| 20 | 20 | <0.1 | 15 | 0.5 |
| 35 | 30 | <0.1 | 25 | 1 |
| 62 | 60 | <0.1 | 50 | 1 |
| 30% Salinity | | | | |
| Control | <0.5 | <0.1 | <0.5 | <0.1 |
| 11 | 10 | <0.1 | 10 | <0.1 |
| 20 | 20 | <0.1 | 15 | <0.1 |
| 35 | 30 | <0.1 | 20 | 1 |
| 62 | 50 | <0.1 | 40 | 2 |
| 109 | 90 | <0.1 | 70 | 1 |

**Table 28 Chemical analyses of model solutions containing chromium(VI) in 20‰ and 30‰ salinity**

NB : See section 5.2.3 for explanation of terms and data

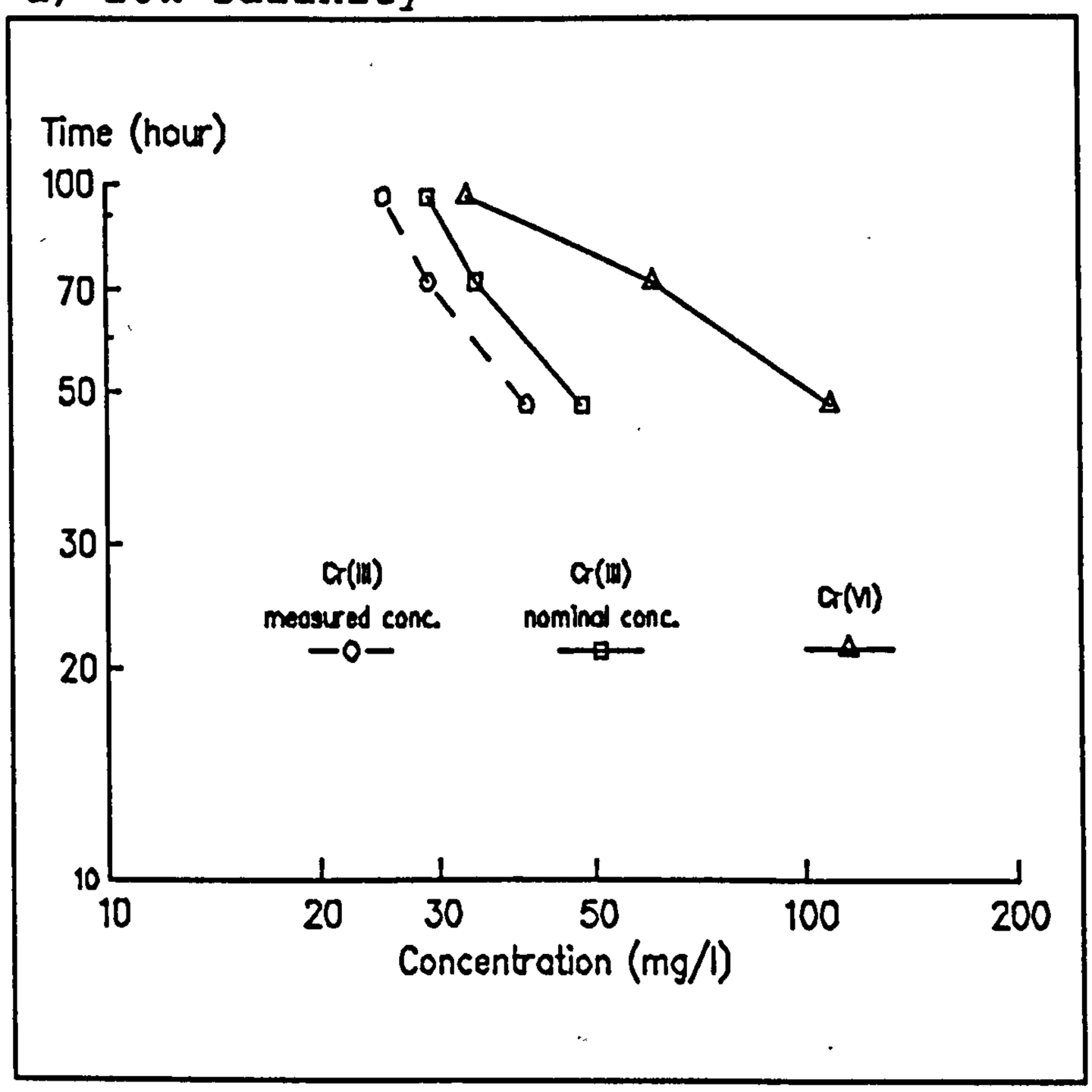
| Conc ($mgl^{-1}$) | Measured Concentration (mg/l) | | | |
|---|---|---|---|---|
| | 0 hr | | 24 hr | |
| | Cr(III) | Cr(VI) | Cr(III) | Cr(VI) |
| 20‰ Salinity | | | | |
| Control | <0.5 | <0.1 | <0.5 | <0.1 |
| 2.7 | <1 | 3 | <1 | 3 |
| 4.8 | <1 | 5 | <1 | 5 |
| 8.6 | <1 | 9 | <1 | 9 |
| 15 | <1 | 15 | (1) | 15 |
| 27 | ND | 30 | ND | 30 |
| 48 | ND | 50 | ND | 45 |
| 86 | ND | 80 | ND | 90 |
| 150 | ND | 140 | ND | 170 |
| 30‰ Salinity | | | | |
| Control | <0.5 | <0.1 | <0.5 | <0.1 |
| 15 | <1 | 15 | <1 | 20 |
| 27 | ND | 30 | ND | 40 |
| 48 | ND | 40 | ND | 50 |
| 86 | ND | 90 | ND | 100 |
| 150 | ND | 160 | ND | 160 |
| 270 | ND | 230 | ND | 220 |

**Table 29 Derived LC50s of chromium(III) and (VI) to *Crangon crangon* in 20‰ and 30‰ salinity**

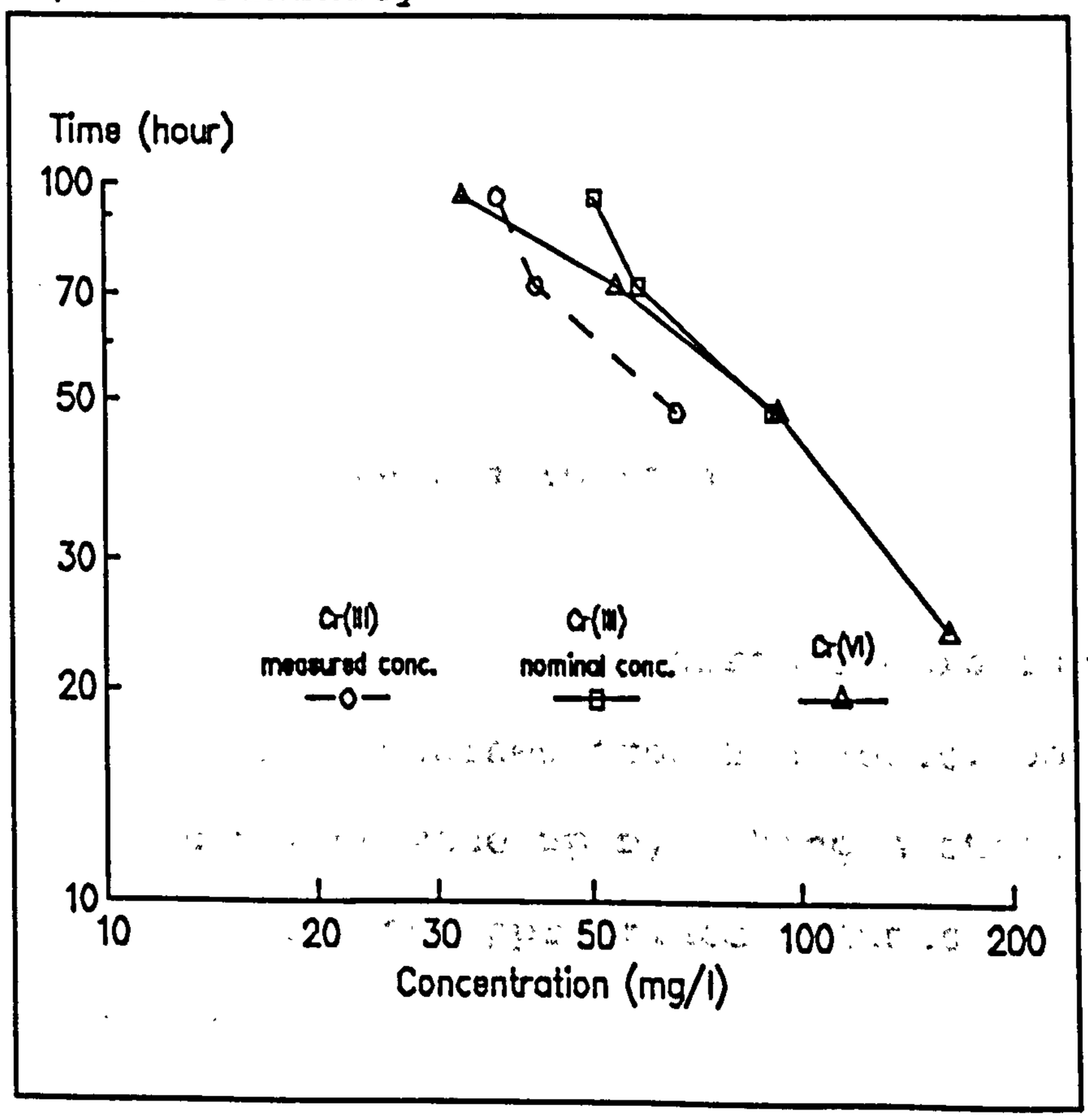
| | Derived LC50s ($mgl^{-1}$) based on nominal concentrations | | | |
|---|---|---|---|---|
| | 24 hr | 48 hr | 72 hr | 96 hr |
| chromium(III) in 20‰ salinity | - | 48 (43-53) | 34 (29-40) | 29 (25-35) |
| chromium(III) in 30‰ salinity | - | 92 (74-154) | 59 (50-72) | 51 (43-60) |
| chromium(VI) in 20‰ salinity | - | 109 (88-147) | 61 (50-77) | 33 (25-44) |
| chromium(VI) in 30‰ salinity | 163 (144-188) | 94 (79-113) | 55 (43-68) | 33 (28-39) |

**Figure 26 Concentration response relationship of *Crangon crangon* to chromium(III) and (VI) at 20‰ and 30‰ salinity**

a) 20‰ salinity




b) 30‰ salinity



## 5.3 TOXICITY OF CHROMIUM(III) AND (VI) TO *Cyprinodon variegatus*, IN SEAWATER

The test chosen is known as an early-lifestage toxicity test, ELS, and has been developed in response to a need in chemical product registration and effluent testing, for a more sensitive test than the 96hr LC50 test to juvenile and adult fish. The principal advantage for this study is that the species used, the sheepshead minnow, is an estuarine species.

The effects monitored included survival, and growth, as dry weight, over a 7 day exposure period. The results are expressed either as the median lethal concentration, LC50, or a no observed effect concentration, NOEC.

The data reported here have been reported as an ICI Group Environmental report BL3924/B, (106)

### 5.3.1 Materials and methods

#### 5.3.1.1 Chemicals and test solutions

The two chromium salts used, were chromic chloride and potassium dichromate, obtained from BDH Poole, Dorset. Exposure solutions were made up by making a stock solution in seawater, and diluting appropriate volumes of these stocks in seawater.

### 5.3.1.2 Toxicity test methods

Ten fish larvae, less than 24 hours old, were put into a one litre glass beaker with the test solution. At each exposure concentration, two duplicate beakers were set up. Surplus larvae were sacrificed and an initial dry weight obtained.

Each day of the test the beakers were then partially emptied, by siphoning off the solution into a second beaker. The new test solution was then added slowly, to avoid undue disturbance to the larvae.

During the test, the larvae were fed *Artemia* nauplii, at a density of 0.0125 g $ml^{-1}$. The excess and dead food was siphoned of at the same time as the renewal of the test solutions.

The survival of the larvae, and any behavioural or developmental abnormalities were checked and recorded daily. A control exposure was run at the same time constituting two vessels with ten larvae in seawater.

At the end of the test the number of larvae surviving were counted, dried and weighed. From the recorded deaths at the respective times, the LC50, (median lethal concentration), at those times, and their confidence intervals were calculated using the method of Stephan, (105). Based on statistical analysis, (ANOVA), of the 7

day survival and growth data, (107), the NOEC, (no observable effect concentration), and LOEC, (lowest observable effect concentration), were obtained in the studies.

#### 5.3.1.3 Physical and chemical analyses

The pH, dissolved oxygen and temperature values were measured in each of the vessels, before and after the test solutions were renewed.

Model solutions of the two chromium salts, at the respective nominal concentrations were also analyzed, using the procedure described in Chapter 3. Total chromium was also measured using direct nebulisation into an ICP-OES instrument during the chromium(VI) study.

### 5.3.2 Results

The survival and growth data for the chromium(III) and (VI) tests are shown in table 30.

The physical parameters of the dilution water used each day in the chromium (III) study, had a pH range of 8.22 - 8.30 and a salinity (‰) of 35.01 - 35.16. In the chromium(VI) study, the dilution water used each day had a pH range of 8.02 - 8.08 and a salinity (‰) of 34.33 - 35.02.

The total chromium, determinations in the chromium(VI) study, obtained during the study are given in table 31, and the results of the analysis of the model solutions are shown in table 32.

A summary of the derived LC50s, with 95% confidence limits, and the NOEC for chromium(III) and (VI) based on growth and survival are presented in table 33.

### 5.3.3 Discussion

The MINEQL data as discussed previously indicated that the majority of chromium(III) was present as $Cr(OH)_4^-$, and that this would precipitate out of solution at the higher concentrations. The analytical data of the model solutions support this prediction. However, for this organism and test, the lowering of the exposure concentrations had little impact on the toxicity results. This may be seen by examining the survival and growth data in table 30, which clearly demonstrates an effect on the organisms at the top concentration, but not at the lower concentrations. The concentration response, figure 27 shows that there is no change in toxicity with time for chromium(III). This data appears to support that found before, section 5.2, for *Crangon crangon*, *i.e.* there is an immediate toxic effect but that possibly because of a lack of mobility, chromium(III) is unable to enter the body of the test animals, thus leading to no longer term effect.

This is in direct contrast to the response obtained with chromium(VI), which as with *Crangon crangon*, was initially less toxic than chromium(III), but with time a toxic effect was noted at increasingly lower concentrations. As noted in section 5.2.3, analysis of chromium(VI) model solutions was very difficult, and the comments made there apply to these test solutions. The test solutions described as ND, *i.e.* chromium(III) not determined, in table 30, suffered from interference by chromium(VI).

**Table 30 Survival and growth of larvae exposed to chromium(III) and (VI)**

| Nom. Conc. (mg/l) | Cumulative Mortality (day) | | | | | | | 7-day % surv. | Mean Dry Weight (mg) |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| Chromium(III) | | | | | | | | | |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0.866 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0.883 |
| 11 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 95 | 0.967 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 1.013 |
| 35 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 95 | 0.899 |
| 63 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 0* | - |
| Chromium(VI) | | | | | | | | | |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0.815 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0.49* |
| 18 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 95 | 0.503* |
| 32 | 0 | 0 | 4 | 7 | 9 | 9 | 11 | 45* | 0.435* |
| 56 | 0 | 7 | 19 | 20 | 20 | 20 | 20 | 0* | - |
| 100 | 12 | 20 | 20 | 20 | 20 | 20 | 20 | 0* | - |

* - significant difference at 5% level

**Table 31 Chemical analyses of chromium(VI) test solutions by ICP-OES**

| Nominal Conc. ($mgl^{-1}$) | Measured concentration ($mgl^{-1}$) | | | |
|---|---|---|---|---|
| | Day 0-1 | Day 3-4 | Day 6-7 | Mean (new & old) |
| 10 (new) | 10.6 | 10.3 | 11.3 | 10.8 |
| (old) | 10.7 | 10.7 | 11.5 | |
| 18 (new) | 18.7 | 19.1 | 19.1 | 19.1 |
| (old) | 18.9 | 19.5 | 19.2 | |
| 32 (new) | 31.6 | 32.9 | 33.3 | 32.9 |
| (old) | 33 | 32.7 | 33.5 | |
| 56 (new) | 55.9 | 56.7 | - | 56.2 |
| (old) | 56.8 | 55.5 | - | |
| 100 (new) | 98.2 | - | - | 97.8 |
| (old) | 97.4 | - | - | |

Seawater control <0.01 $mgl^{-1}$

**Table 32 Chemical analyses of model solutions containing chromium(III) and (VI) in seawater**

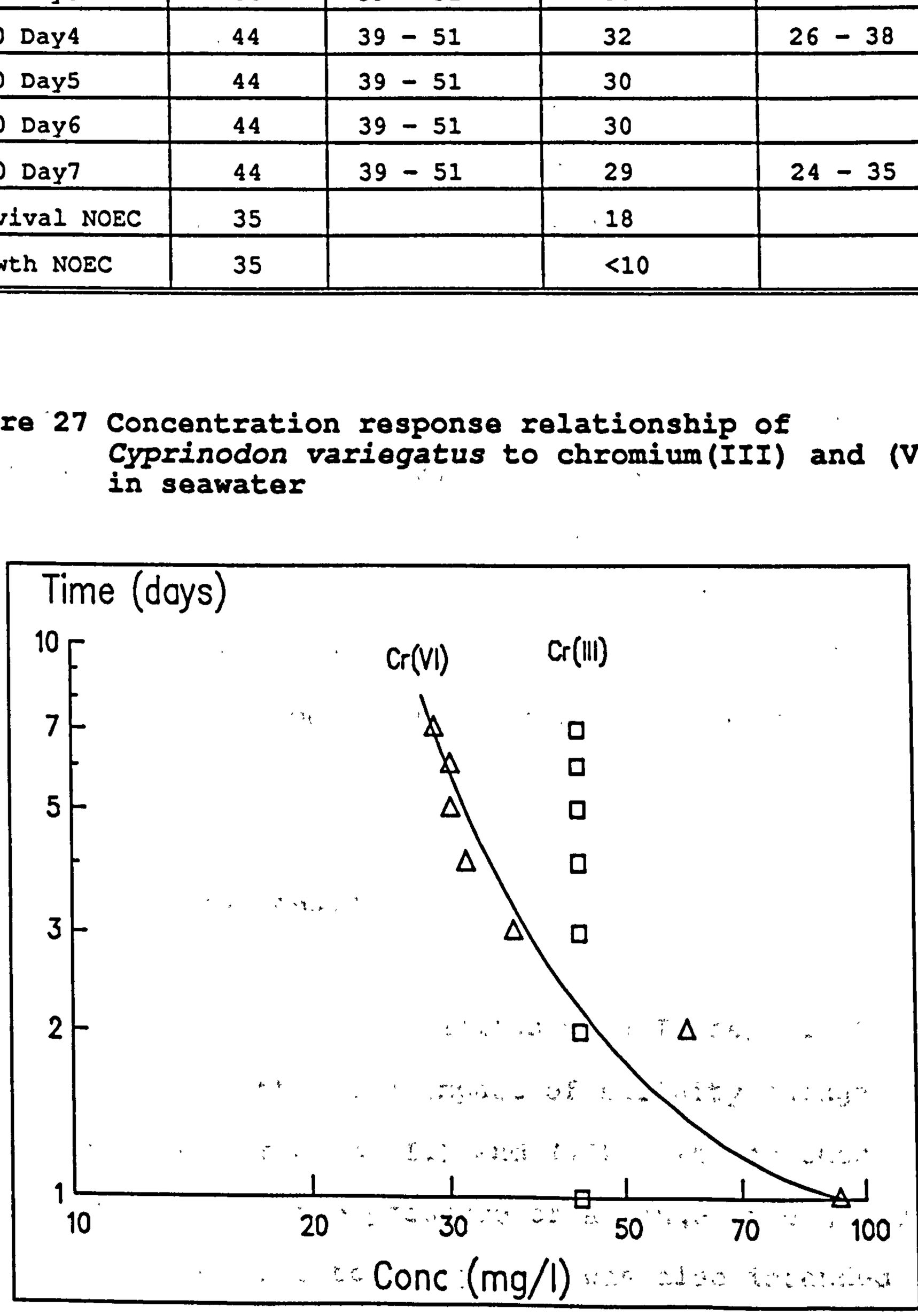
NB : See section 5.3.3 for explanation of terms and data

| Conc ($mgl^{-1}$) | Measured Concentration (mg/l) | | | |
|---|---|---|---|---|
| | 0 hr | | 24 hr | |
| | Cr(III) | Cr(VI) | Cr(III) | Cr(VI) |
| Chromium(III) solutions | | | | |
| 6 | 5.8 | <0.1 | 5.4 | <0.1 |
| 11 | 10 | <0.1 | 9 | <0.1 |
| 20 | 19 | <0.1 | 15 | 1 |
| 35 | 30 | <0.1 | 20 | <0.1 |
| 63 | 60 | <0.1 | 40 | 1 |
| Chromium(VI) solutions | | | | |
| 10 | <1 | 10 | <1 | 15 |
| 18 | ND | 15 | ND | 20 |
| 32 | ND | 30 | ND | 30 |
| 56 | ND | 60 | ND | 70 |
| 100 | ND | 110 | ND | 90 |

**Table 33 Derived LC50s and NOECs of chromium(III) and (VI) to *Cyprinodon variegatus* in seawater**

| Parameter | Chromium(III) | | Chromium(VI) | |
|---|---|---|---|---|
| | Value ($mgl^{-1}$) | Conf. Limits ($mgl^{-1}$) | Value ($mgl^{-1}$) | Conf. Limits ($mgl^{-1}$) |
| LC50 Day1 | 44 | 39 - 51 | 93 | |
| LC50 Day2 | 44 | 39 - 51 | 60 | |
| LC50 Day3 | 44 | 39 - 51 | 36 | |
| LC50 Day4 | 44 | 39 - 51 | 32 | 26 - 38 |
| LC50 Day5 | 44 | 39 - 51 | 30 | |
| LC50 Day6 | 44 | 39 - 51 | 30 | |
| LC50 Day7 | 44 | 39 - 51 | 29 | 24 - 35 |
| Survival NOEC | 35 | | 18 | |
| Growth NOEC | 35 | | <10 | |

**Figure 27 Concentration response relationship of *Cyprinodon variegatus* to chromium(III) and (VI) in seawater**



## 5.4 TOXICITY OF CHROMIUM(III) AND (VI) TO *Tisbe bataglíai* IN ESTUARINE CONDITIONS

*Tisbe* is a marine benthic copepod, which having a wide geographic distribution is an ideal animal for toxicity tests. Due to its short lifecycle and size, a number of benefits are obtained. First, the exposure is relatively short, lasting 48 hours, which makes it possible to run a number of tests consecutively, amending the following studies, based on the results of the previous work. Secondly, because the animals are small, the test vessels and test solutions are also small. Thus the test is carried out in small 20 ml vials, with 15 ml of sample. This means that it is possible to set up a large number of exposures, and a number of variables are easily investigated. Finally a sensitive stage of the lifecycle could be easily identified, and hence used to test at concentrations of chromium closer to those that will be found in the environment, than was possible with other organisms.

### 5.4.1 Experimental design

The object of these investigations with *Tisbe*, was first to investigate further the impact of salinity changes on the toxicity of chromium(III) and (VI). It was then intended to see how the presence of a range of organic ligands affected that toxicity. It was also intended to investigate whether the observed toxicity mirrored the

predictions in chromium speciation from MINEQL, or observed changes in the available chromium species, as measured by electrochemistry.

The experiments were originally to be run in 3 groups, as described in table 34. However, as discussed later the second and third sets were amended by leaving out the 10‰ salinity experiments, and then amalgamated and run as one set.

The initial experiments were aimed at defining the toxicity of chromium(III) and (VI) in 10, 20 and 30 ‰ salinity. This would thus be a base line against which toxicity modifications would be compared. The observed toxicity changes with salinity would also be useful, in highlighting any differences between the two oxidation states in estuarine waters.

As originally anticipated the second and third series of experiments, would rely on the results from the first to set the concentrations to be run. To these test solutions would be added a number of organic ligands, which were thought to alter the availability of chromium(III), and might interact with chromium(VI), and give rise to possible changes in speciation of the chromium. The concentration of chromium(VI) was chosen on the basis of being the lowest concentration at which little or no toxicity occurred.

**Table 34 Experimental design for exposure of *Tisbe bataglíai* to chromium(III) and (VI) in model estuarine waters.**

| Chromium oxidation state | Test concentrations, and organic ligands | | | Water salinity (‰) |
|---|---|---|---|---|
| SET 1 | | | | |
| Cr(III) | 0.05, 0.1, 0.2, 0.4, 0.8 $mgl^{-1}$ | | | 10 |
| Cr(III) | 0.1, 0.2, 0.4, 0.8, 1.6 $mgl^{-1}$ | | | 20 |
| Cr(III) | 0.2, 0.4, 0.8, 1.6, 3.2 $mgl^{-1}$ | | | 30 |
| Cr(VI) | 0.05, 0.1, 0.2, 0.4, 0.8 $mgl^{-1}$ | | | 10 |
| Cr(VI) | 0.1, 0.2, 0.4, 0.8, 1.6 $mgl^{-1}$ | | | 20 |
| Cr(VI) | 0.2, 0.4, 0.8, 1.6, 3.2 $mgl^{-1}$ | | | 30 |
| SET 2 | times lowest 100% effect conc. at 48 hour | Organic ligand | %complexed* | |
| Cr(III) | 1 | EDTA | 100 | 10,20,30 |
| Cr(III) | 5 | EDTA | 80 | 10,20,30 |
| Cr(III) | 1 | NTA | 100 | 10,20,30 |
| Cr(III) | 5 | NTA | 80 | 10,20,30 |
| Cr(III) | 1 | Citric acid | 100 | 10,20,30 |
| Cr(III) | 2 | Citric acid | 50 | 10,20,30 |
| SET 3 | Conc of Cr(VI) (mg $l^{-1}$) | Organic ligand | Conc (mg $l^{-1}$) | |
| Cr(VI) | 0.5 | EDTA | 0.3 | 10,20,30 |
| Cr(VI) | 0.5 | EDTA | 1.2 | 10,20,30 |
| Cr(VI) | 0.5 | NTA | 0.15 | 10,20,30 |
| Cr(VI) | 0.5 | NTA | 0.6 | 10,20,30 |
| Cr(VI) | 0.5 | Citric acid | 0.15 | 10,20,30 |
| Cr(VI) | 0.5 | Citric acid | 0.6 | 10,20,30 |

* : Calculated thermodynamically

**NB : The 10‰ salinity tests were not run for Sets 2 or 3, see section 5.4.4.1**

### 5.4.2 Materials and methods

#### 5.4.2.1 Chemicals and test solutions

The two chromium salts used, were chromic chloride and potassium dichloride, obtained from BDH Poole, Dorset. Exposure solutions were prepared by making a stock solution in MilliQ water of 100 mg $l^{-1}$, and diluting aliquots of the stock in water of appropriate salinity.

In the two sets of experiments when organic ligands were added, this method of making up test solutions was modified, such that the chromium was prepared as a secondary stock in water of 10, 20 or 30‰ salinity. This was then diluted with the test medium to which the organic chelator had already been added. This was necessary to ensure the water was at the correct salinity, and that the organic chelator was not being added at concentrations in excess of its solubility.

#### 5.4.2.2 Toxicity test methods

Nauplii, <48 hours old were used in the test, with 15 animals at each concentration, five each in three disposable polystyrene tissue culture cells. Mortality of the *Tisbe* was assessed at 24 and 48 hours by microscopy.

From the recorded deaths at 24 and 48 hours, the LC50 at those times, and their confidence limits were calculated

using the method of Stephan, (105).

### 5.4.2.3 Physical and chemical analyses

The pH, dissolved oxygen, salinity and temperature values were measured in the bulk test media at the time of making up the test solutions.

The test solutions were analysed at 0 hours for total chromium for confirmation of the concentrations, and at 24 and 48 hours for chromium(III) and (VI) using the methods described in sections 3.8 and 3.9. Aliquots of the excess solutions, held in the original containers, were used for these determinations.

## 5.4.3 Results

The physical parameters of the seawater used to make up the test solutions had a pH range of 8.10 - 8.25, dissolved oxygen of 8.8 - 9.1 mg, and a salinity, (‰), of 34.9 - 35.1.

The survival data for the chromium(III) and (VI) tests in 10, 20 or 30‰ salinity are shown in table 35. The results of the chromium determinations carried out in support of these tests are shown in table 36.

The derived EC50 values are presented in table 37 for the chromium studies.

Figure 28 shows the mortality and measured chromium data at 20 and 30‰ salinity.

The survival data for the chromium(III) and (VI) tests in 20 or 30‰ salinity in the presence of organic chelators are shown in table 38. The results of the chromium determinations carried out in support of these tests are shown in table 39.

The combined mortality and measured chromium concentrations are displayed in figures 29 -34. These figures include data from the first run for comparison. The data chosen were those closest to the concentrations being run, *i.e.* 0.4 and 1.6 mg $l^{-1}$, compared to 0.4 and 2.0 mg $l^{-1}$ in the organic ligand study, for chromium(III), and similarly 0.4 compared to 0.5 mg $l^{-1}$ chromium(VI).

### 5.4.4 Discussion

#### 5.4.4.1 10‰ salinity

No animals survived at 10‰ salinity even in the control exposure. Given the survival of the control animals at 20‰ salinity, this suggests that the LC50 for the effect of salinity to *Tisbe* lies between 10 and 20‰. Although not recorded before, most workers have tended to limit the working range to 20‰ salinity. It would be useful to have an LC50 for salinity to *Tisbe*, and this is an experiment that should be carried out.

Consequently it was only possible to investigate the effect of chromium(III) and (VI) to *Tisbe* at 20 and 30‰ salinity. These data will be discussed under the relevant chromium oxidation state, sections 5.4.4.2 and 5.4.4.3.

**Table 35 Percent mortality of *Tisbe battagliai* exposed to chromium(III) and (VI) at different salinities**

| Test conc. (mg/l) | % Mortalities | | | | | |
|---|---|---|---|---|---|---|
| | 10‰ salinity+ | | 20‰ salinity | | 30‰ salinity | |
| | 24 hr | 48 hr | 24 hr | 48 hr | 24 hr | 48 hr |
| Control* | 100 | 100 | 0 | 0 | 0 | 0 |
| Chromium(III) | | | | | | |
| 0.05 | 100 | 100 | - | - | - | - |
| 0.1 | 100 | 100 | 0 | 20 | - | - |
| 0.2 | 100 | 100 | 0 | 53 | 0 | 40 |
| 0.4 | 100 | 100 | 7 | 67 | 7 | 80 |
| 0.8 | - | - | 33 | 87# | 13 | 80# |
| 1.6 | - | - | 47 | 87# | 27 | 87# |
| 3.2 | - | - | - | - | 0# | 20# |
| Chromium(VI) | | | | | | |
| 0.05 | 100 | 100 | - | - | - | - |
| 0.1 | 100 | 100 | 0 | 0 | - | - |
| 0.2 | 100 | 100 | 0 | 0 | 0 | 0 |
| 0.4 | 100 | 100 | 0 | 0 | 0 | 7 |
| 0.8 | - | - | 0 | 40 | 0 | 7 |
| 1.6 | - | - | 0 | 60 | 0 | 53 |
| 3.2 | - | - | - | - | 0 | 100 |

+ : At all concentrations the animals were dead within 10 minutes

* : The control was common to chromium(III) and (VI)

# : Particulates noticed in the test vessels, see section 5.4.4

**Table 36 Chemical analyses of test solutions containing chromium(III) or (VI) in 20 and 30‰ salinity**

| Test conc. (mg/l) | Measured concentration (mg/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 20‰ salinity | | | | 30‰ salinity | | | |
| | Cr(III) | | Cr(VI) | | Cr(III) | | Cr(VI) | |
| | 24hr | 48hr | 24hr | 48hr | 24hr | 48hr | 24hr | 48hr |
| Control* | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Chromium(III) | | | | | | | | |
| 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | - | - | - | - |
| 0.2 | 0.2 | 0.2 | <0.1 | <0.1 | 0.2 | 0.3 | <0.1 | <0.1 |
| 0.4 | 0.35 | 0.2 | <0.1 | <0.1 | 0.5 | 0.4 | <0.1 | <0.1 |
| 0.8 | 0.9 | 0.6# | <0.1 | <0.1# | 0.7 | 0.8# | <0.1 | <0.1# |
| 1.6 | 1.8 | 1.6# | <0.1 | <0.1# | 1.2 | 1.9# | <0.1 | <0.1# |
| 3.2 | - | - | - | - | 2.7# | 2.9# | <0.1# | <0.1# |
| Chromium(VI) | | | | | | | | |
| 0.1 | <0.2 | <0.2 | 0.1 | 0.1 | - | - | - | - |
| 0.2 | <0.2 | <0.2 | 0.2 | 0.1 | <0.2 | <0.2 | 0.2 | 0.2 |
| 0.4 | <0.2 | <0.2 | 0.4 | 0.5 | <0.2 | <0.2 | 0.3 | 0.4 |
| 0.8 | <0.2 | <0.2 | 0.8 | 0.9 | <0.2 | <0.2 | 0.7 | 0.9 |
| 1.6 | <0.2 | <0.2 | 1.4 | 1.4 | <0.2 | <0.2 | 1.7 | 1.6 |
| 3.2 | - | - | - | - | <0.2 | <0.2 | 3.0 | 3.3 |

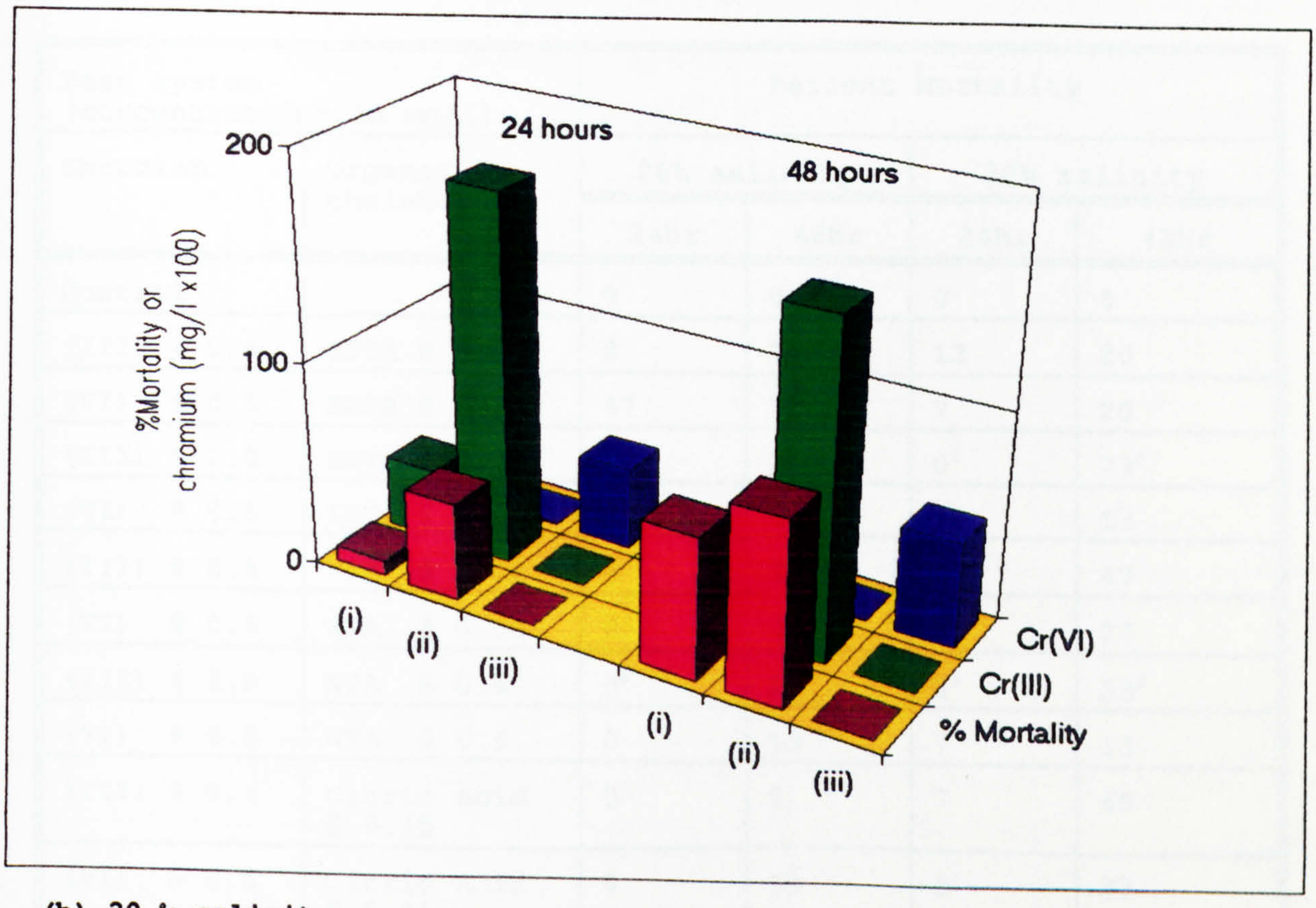
* : The control was common to chromium(III) and (VI)
\# : Particulates noticed in the test vessels, see section 5.4.4.2

**Table 37 Derived LC50s of chromium(III) and (VI) to *Tisbe bataglíai* in 20‰ and 30‰ salinity at 48 hours**

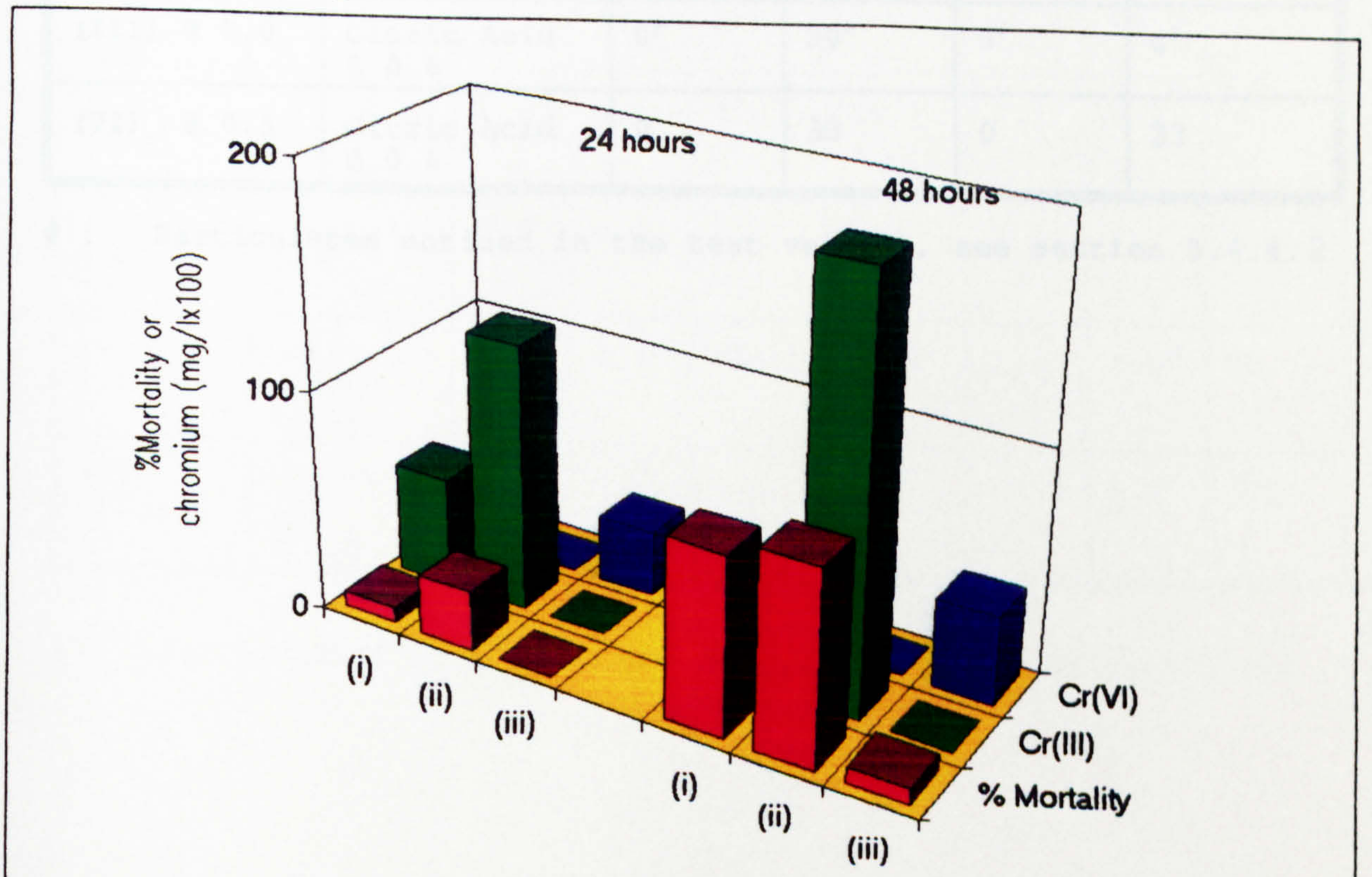
| | Derived LC50 - nominal conc. (mg $l^{-1}$) | Derived LC50 measured conc. (mg $l^{-1}$) |
|---|---|---|
| chromium(III) in 20‰ salinity | 0.22 | 0.22 |
| chromium(III) in 30‰ salinity | 0.21 | 0.29 |
| chromium(VI) in 20‰ salinity | 1.2 | 1.1 |
| chromium(VI) in 30‰ salinity | 1.4 | 1.4 |

**Figure 28 Toxicity of chromium to *Tisbe bataglias***

**(a) 20 ‰ salinity**




**(b) 30 ‰ salinity**




**Test concentrations**
**(i) : 0.4 mg $l^{-1}$ chromium(III)**
**(ii) : 1.6 mg $l^{-1}$ chromium(III)**
**(iii) : 0.4 mg $l^{-1}$ chromium(VI)**

**Table 38 Percent mortality of *Tisbe battagliai* exposed to chromium(III) and (VI) at different salinities in the presence of organic chelators**

| Test system (concentrations in mg/l) | | Percent Mortality | | | |
|---|---|---|---|---|---|
| Chromium | Organic chelator | 20‰ salinity | | 30‰ salinity | |
| | | 24hr | 48hr | 24hr | 48hr |
| Control | | 0 | 0 | 0 | 0 |
| (III) @ 0.4 | EDTA @ 0.3 | 0 | 7 | 13 | 20 |
| (VI) @ 0.5 | EDTA @ 0.3 | 47 | 93 | 7 | 20 |
| (III) @ 2.0 | EDTA @ 1.2 | 7# | 7# | 0# | 73# |
| (VI) @ 0.5 | EDTA @ 1.2 | 20 | 33 | 0 | 13 |
| (III) @ 0.4 | NTA @ 0.15 | 0 | 33 | 27 | 47 |
| (VI) @ 0.5 | NTA @ 0.15 | 0 | 40 | 0 | 27 |
| (III) @ 2.0 | NTA @ 0.6 | 0# | 27# | 0# | 33# |
| (VI) @ 0.5 | NTA @ 0.6 | 0 | 53 | 7 | 13 |
| (III) @ 0.4 | Citric Acid @ 0.15 | 0 | 0 | 7 | 60 |
| (VI) @ 0.5 | Citric Acid @ 0.15 | 0 | 33 | 0 | 33 |
| (III) @ 2.0 | Citric Acid @ 0.6 | 0# | 20# | 0# | 0# |
| (VI) @ 0.5 | Citric Acid @ 0.6 | 0 | 33 | 0 | 33 |

# : Particulates noticed in the test vessels, see section 5.4.4.2

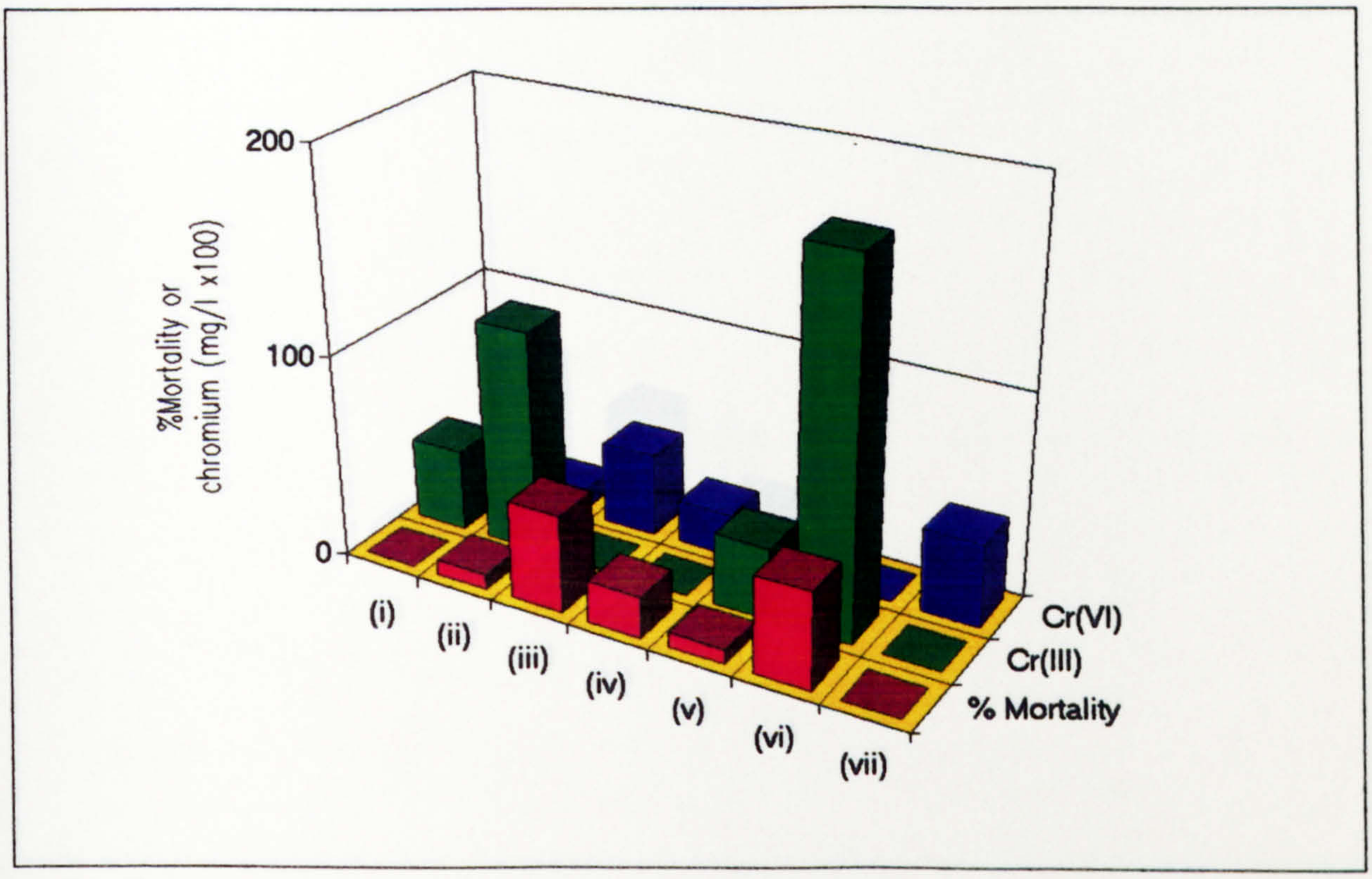
**Table 39 Chemical analyses of test solutions containing chromium(III) or (VI) in 20 and 30‰ salinity**

| Conc. (mg/l) | | Measured concentration (mg/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chromium | Organic chelator | 20‰ salinity | | | | 30‰ salinity | | | |
| | | Cr(III) | | Cr(VI) | | Cr(III) | | Cr(VI) | |
| | | 24hr | 48hr | 24hr | 48hr | 24hr | 48hr | 24hr | 48hr |
| Control | | <0.1 | <0.1 | <0.1 | <0.1 | <0.2 | <0.2 | <0.1 | <0.1 |
| (III) @ 0.4 | EDTA @ 0.3 | 0.4 | 0.5 | <0.1 | <0.1 | 0.2 | 0.2 | <0.1 | <0.1 |
| (VI) @ 0.5 | EDTA @ 0.3 | <0.1 | 0.2 | 0.4 | 0.4 | <0.2 | <0.2 | 0.6 | 0.3 |
| (III) @ 2.0 | EDTA @ 1.2 | 1.1# | 1.8# | 0.1# | 0.1# | 0.9# | 0.9# | <0.1# | <0.1# |
| (VI) @ 0.5 | EDTA @ 1.2 | <0.1 | <0.1 | 0.2 | 0.3 | <0.2 | <0.2 | 0.2 | 0.4 |
| (III) @ 0.4 | NTA @ 0.15 | 0.2 | 0.2 | <0.1 | <0.1 | 0.3 | 0.3 | <0.1 | <0.1 |
| (VI) @ 0.5 | NTA @ 0.15 | <0.1 | <0.1 | 0.4 | 0.6 | <0.2 | <0.2 | 0.4 | 0.4 |
| (III) @ 2.0 | NTA @ 0.6 | 0.3# | 0.5# | <0.1# | <0.1# | 0.6# | 0.8# | <0.1# | <0.1# |
| (VI) @ 0.5 | NTA @ 0.6 | <0.1 | <0.1 | 0.5 | 0.4 | <0.2 | <0.2 | 0.5 | 0.6 |
| (III) @ 0.4 | Citric Acid @ 0.15 | 0.2 | 0.2 | <0.1 | <0.1 | 0.2 | 0.3 | <0.1 | <0.1 |
| (VI) @ 0.5 | Citric Acid @ 0.15 | 0.2 | 0.2 | 0.4 | 0.3 | 0.2 | 0.2 | 0.3 | 0.3 |
| (III) @ 2.0 | Citric Acid @ 0.6 | 0.6# | 0.9# | <0.1# | <0.1# | 1.2# | 1.4# | <0.1# | <0.1# |
| (VI) @ 0.5 | Citric Acid @ 0.6 | 0.1 | 0.2 | 0.4 | 0.3 | <0.2 | 0.2 | 0.3 | 0.2 |

# : Particulates noticed in the test vessels, see section 5.4.4

**Figure 29 Toxicity of chromium to *Tisbe bataglias* in the presence of EDTA in 20% salinity**

**(a) Exposure time = 24 hours**




**(b) Exposure time = 48 hours**

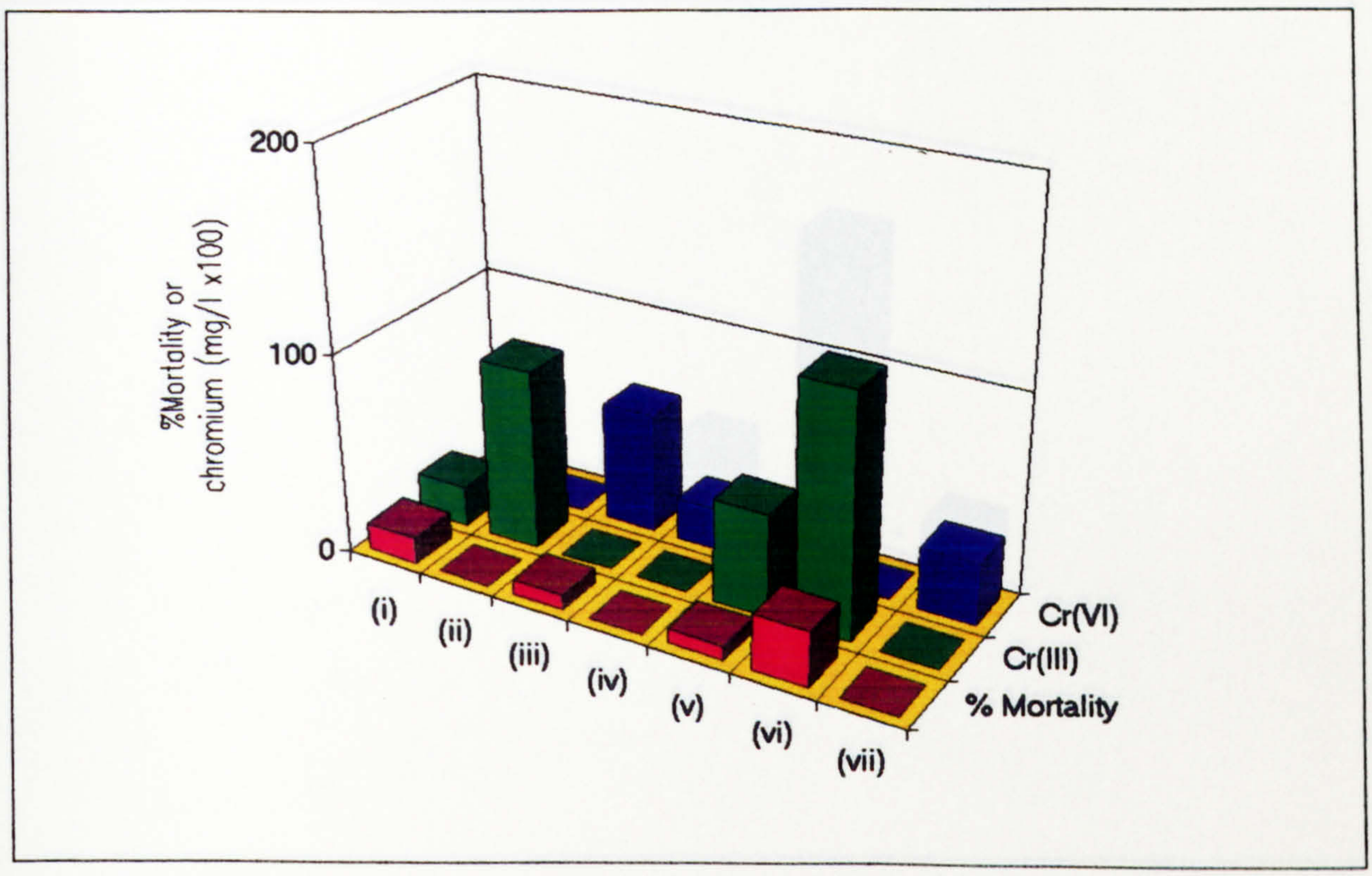



**Test concentrations**

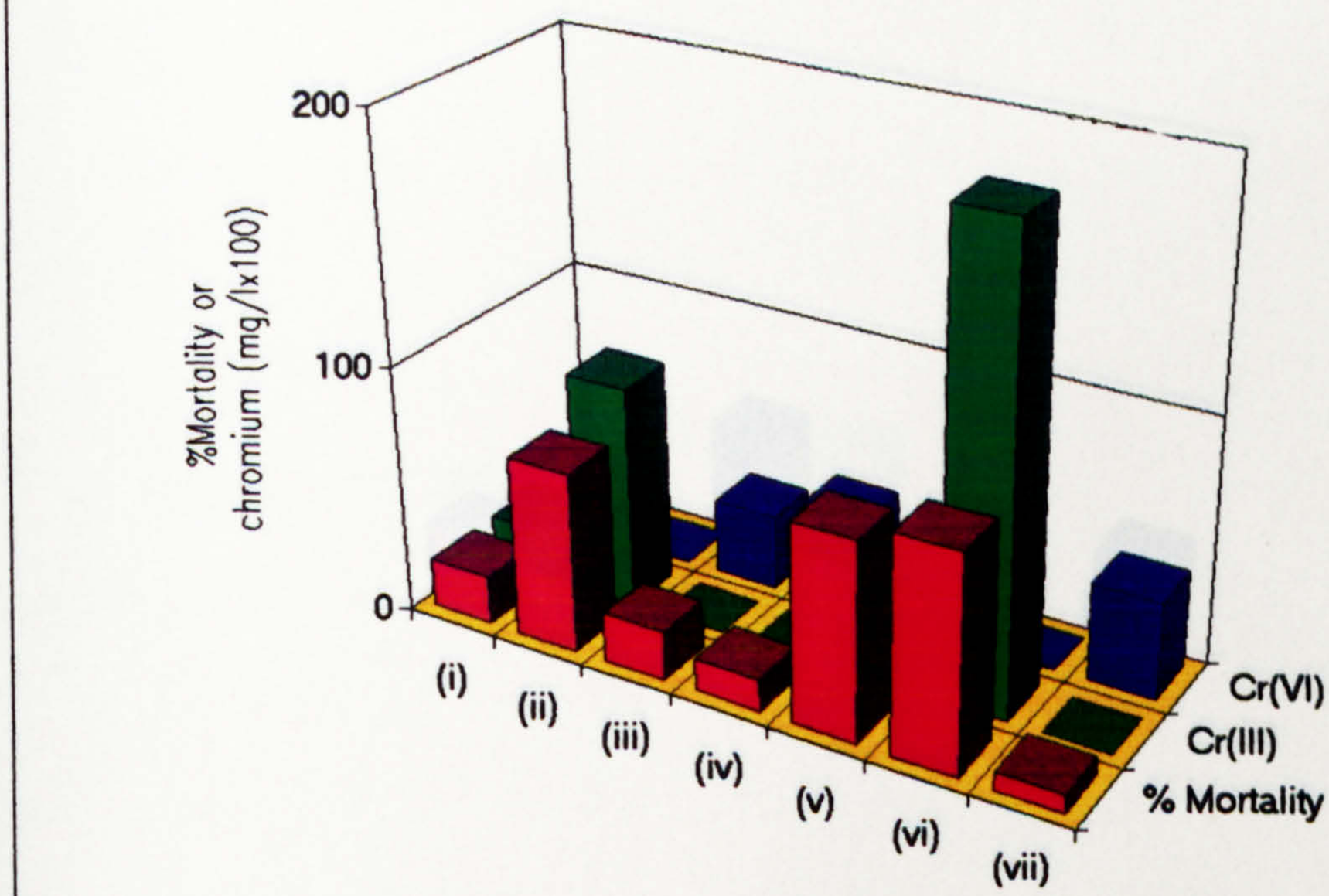
| | | | |
|---|---|---|---|
| (i) | : 0.4 mg $l^{-1}$ Cr(III) + 0.3 mg/l EDTA | (v) | : 0.4 mg $l^{-1}$ Cr(III) |
| (ii) | : 2.0 mg $l^{-1}$ Cr(III) + 1.2 mg/l EDTA | (vi) | : 1.6 mg $l^{-1}$ Cr(III) |
| (iii) | : 0.5 mg $l^{-1}$ Cr(VI) + 0.3 mg/l EDTA | (vii) | : 0.4 mg $l^{-1}$ Cr(VI) |
| (iv) | : 0.5 mg $l^{-1}$ Cr(VI) + 1.2 mg/l EDTA | | |

**Figure 30 Toxicity of chromium to *Tisbe bataglias* in the presence of EDTA in 30% salinity**

**(a) Exposure time = 24 hours**




**(b) Exposure time = 48 hours**

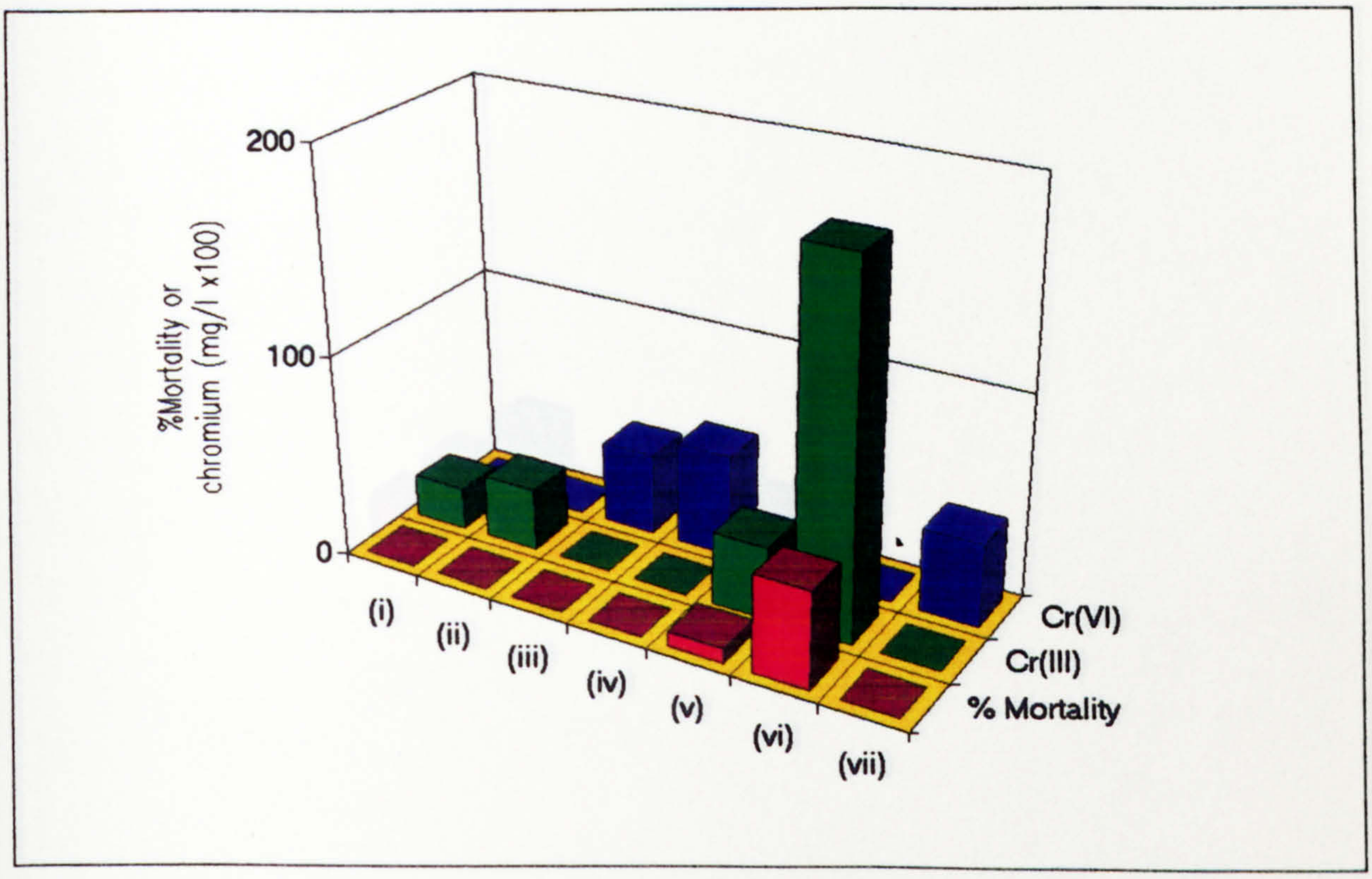



**Test concentrations**

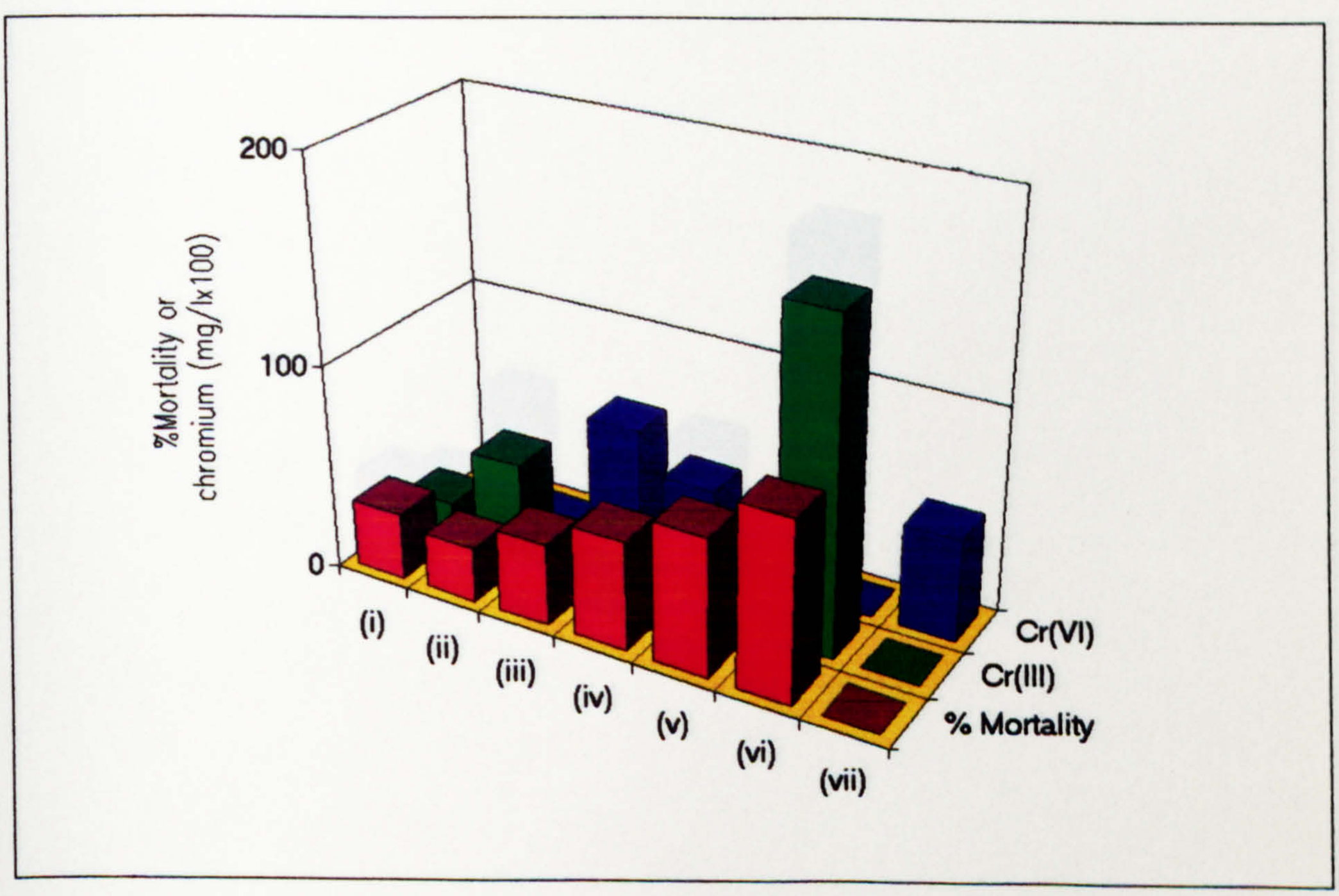
| | | | |
|---|---|---|---|
| (i) | : 0.4 mg $l^{-1}$ Cr(III) + 0.3 mg/l EDTA | (v) | : 0.4 mg $l^{-1}$ Cr(III) |
| (ii) | : 2.0 mg $l^{-1}$ Cr(III) + 1.2 mg/l EDTA | (vi) | : 1.6 mg $l^{-1}$ Cr(III) |
| (iii) | : 0.5 mg $l^{-1}$ Cr(VI) + 0.3 mg/l EDTA | (vii) | : 0.4 mg $l^{-1}$ Cr(VI) |
| (iv) | : 0.5 mg $l^{-1}$ Cr(VI) + 1.2 mg/l EDTA | | |

**Figure 31 Toxicity of chromium to *Tisbe bataglias* in the presence of NTA in 20‰ salinity**

**(a) Exposure time = 24 hours**




**(b) Exposure time = 48 hours**

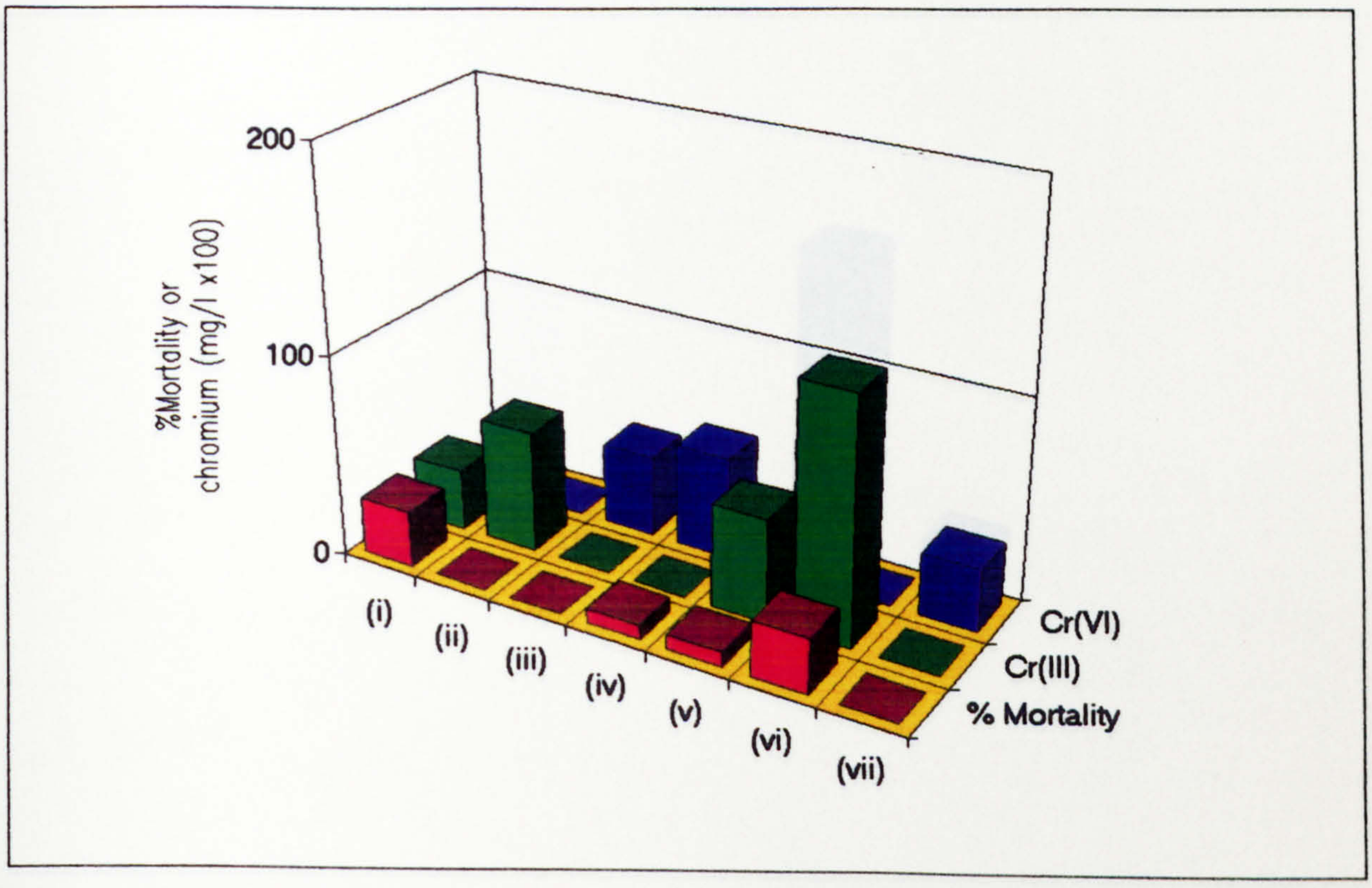



**Test concentrations**

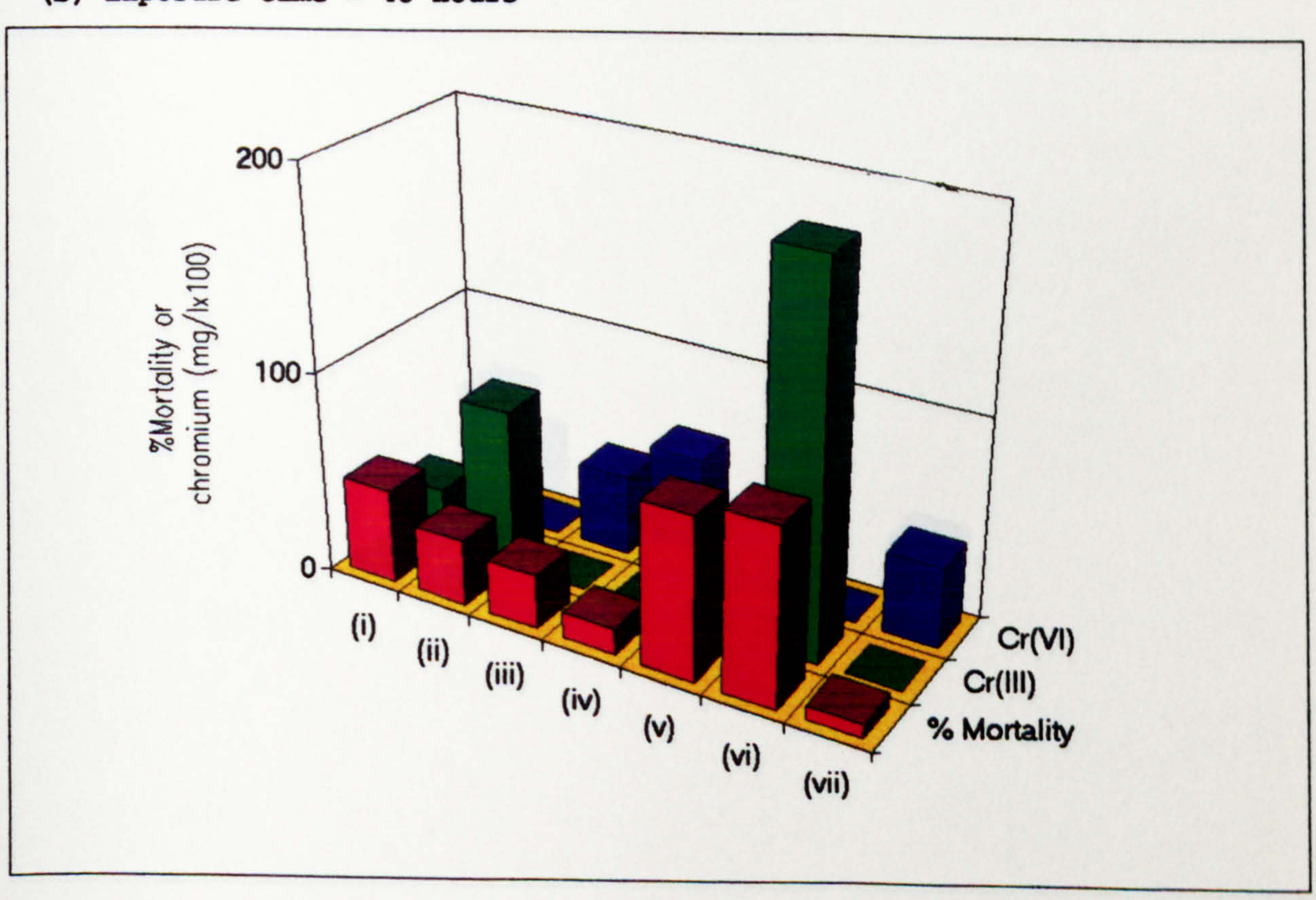
| | | | |
|---|---|---|---|
| **(i)** | **: 0.4 mg $l^{-1}$ Cr(III) + 0.3 mg/l NTA** | **(v)** | **: 0.4 mg $l^{-1}$ Cr(III)** |
| **(ii)** | **: 2.0 mg $l^{-1}$ Cr(III) + 1.2 mg/l NTA** | **(vi)** | **: 1.6 mg $l^{-1}$ Cr(III)** |
| **(iii)** | **: 0.5 mg $l^{-1}$ Cr(VI) + 0.3 mg/l NTA** | **(vii)** | **: 0.4 mg $l^{-1}$ Cr(VI)** |
| **(iv)** | **: 0.5 mg $l^{-1}$ Cr(VI) + 1.2 mg/l NTA** | | |

**Figure 32 Toxicity of chromium to *Tisbe bataglias* in the presence of NTA in 30% salinity**

**(a) Exposure time = 24 hours**




**(b) Exposure time = 48 hours**

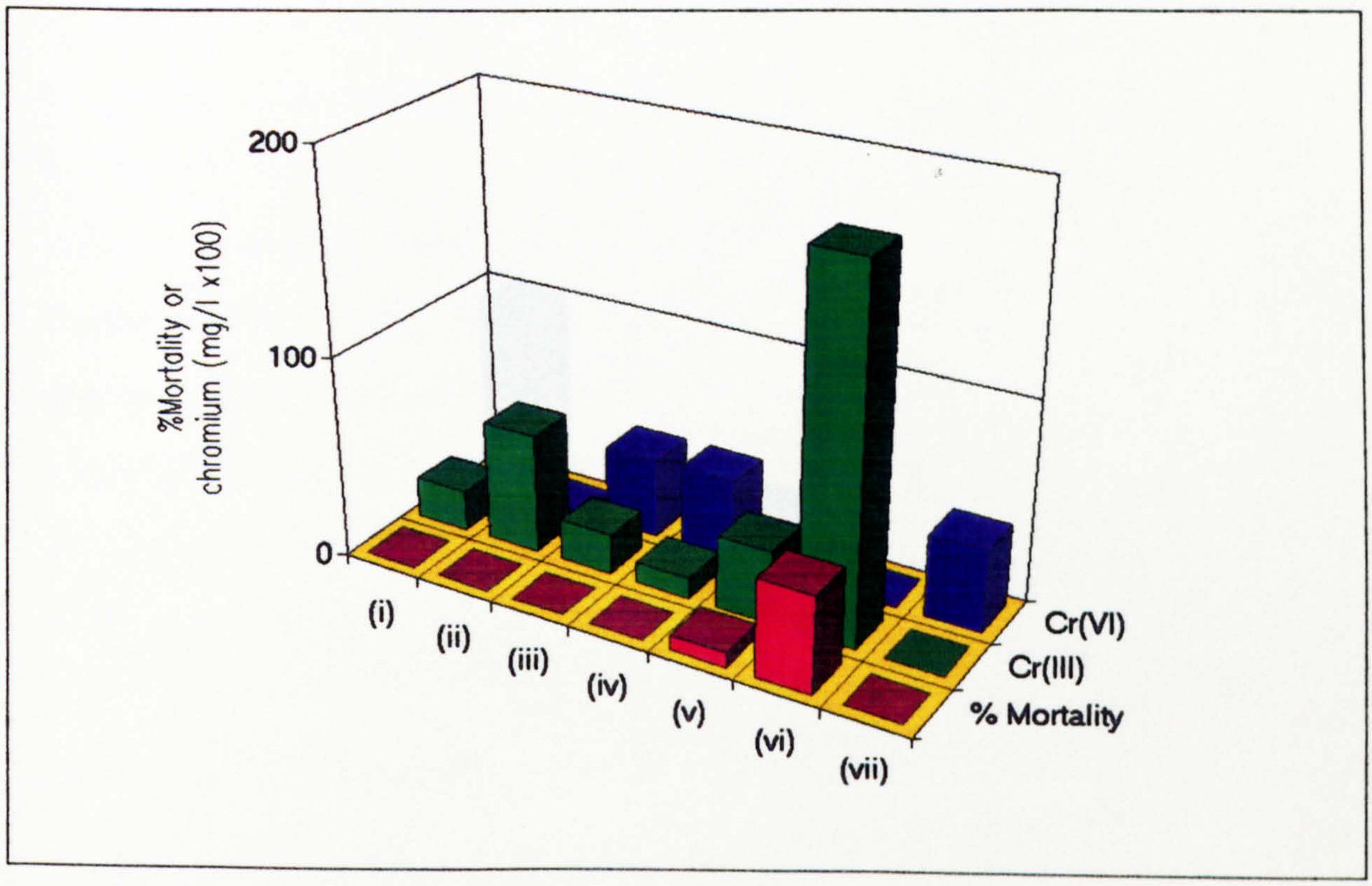



**Test concentrations**

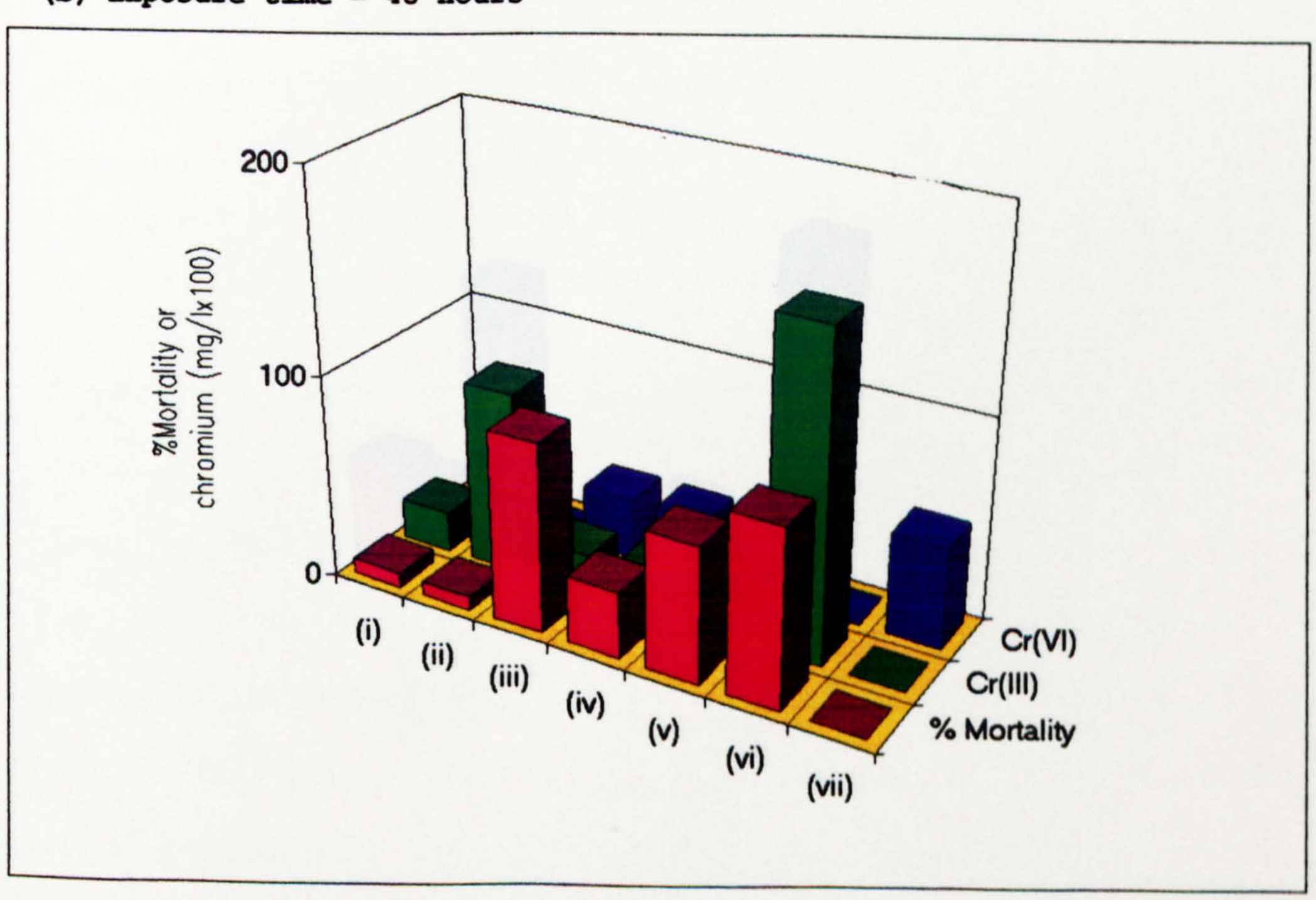
| | | | |
|---|---|---|---|
| (i) | : 0.4 mg $l^{-1}$ Cr(III) + 0.3 mg/l NTA | (v) | : 0.4 mg $l^{-1}$ Cr(III) |
| (ii) | : 2.0 mg $l^{-1}$ Cr(III) + 1.2 mg/l NTA | (vi) | : 1.6 mg $l^{-1}$ Cr(III) |
| (iii) | : 0.5 mg $l^{-1}$ Cr(VI) + 0.3 mg/l NTA | (vii) | : 0.4 mg $l^{-1}$ Cr(VI) |
| (iv) | : 0.5 mg $l^{-1}$ Cr(VI) + 1.2 mg/l NTA | | |

**Figure 33 Toxicity of chromium to *Tisbe bataglias* in the presence of citric acid, (CA), in 20‰ salinity**

**(a) Exposure time = 24 hours**




**(b) Exposure time = 48 hours**

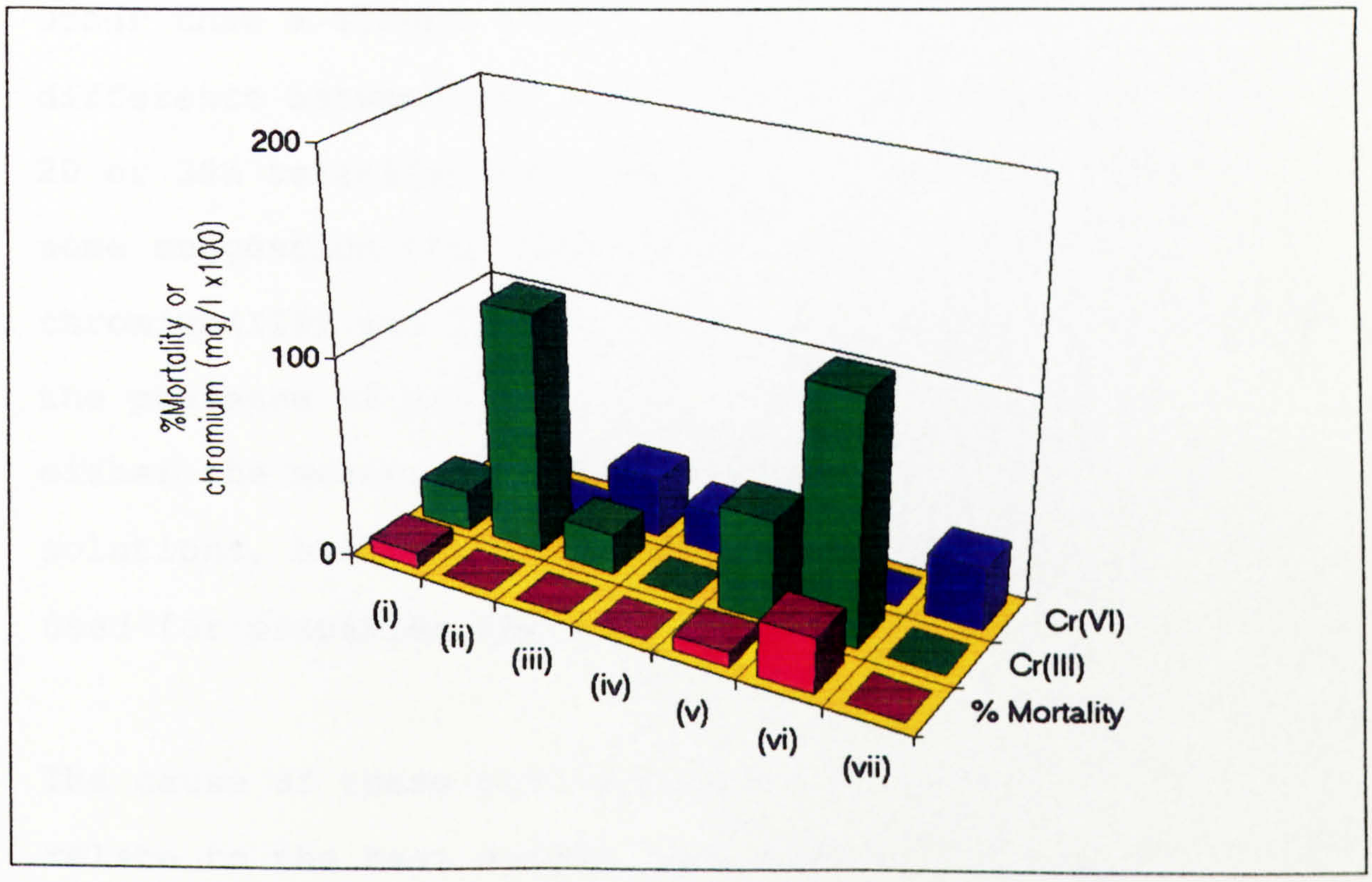



**Test concentrations**

| | | | |
|---|---|---|---|
| (i) | : 0.4 mg $l^{-1}$ Cr(III) + 0.3 mg/l CA | (v) | : 0.4 mg $l^{-1}$ Cr(III) |
| (ii) | : 2.0 mg $l^{-1}$ Cr(III) + 1.2 mg/l CA | (vi) | : 1.6 mg $l^{-1}$ Cr(III) |
| (iii) | : 0.5 mg $l^{-1}$ Cr(VI) + 0.3 mg/l CA | (vii) | : 0.4 mg $l^{-1}$ Cr(VI) |
| (iv) | : 0.5 mg $l^{-1}$ Cr(VI) + 1.2 mg/l CA | | |

**Figure 34 Toxicity of chromium to *Tisbe bataglias* in the presence of citric acid, (CA), in 30‰ salinity**

**(a) Exposure time = 24 hours**




**(b) Exposure time = 48 hours**




**Test concentrations**

| | | | |
|---|---|---|---|
| (i) | : 0.4 mg $l^{-1}$ Cr(III) + 0.3 mg/l CA | (v) | : 0.4 mg $l^{-1}$ Cr(III) |
| (ii) | : 2.0 mg $l^{-1}$ Cr(III) + 1.2 mg/l CA | (vi) | : 1.6 mg $l^{-1}$ Cr(III) |
| (iii) | : 0.5 mg $l^{-1}$ Cr(VI) + 0.3 mg/l CA | (vii) | : 0.4 mg $l^{-1}$ Cr(VI) |
| (iv) | : 0.5 mg $l^{-1}$ Cr(VI) + 1.2 mg/l CA | | |

### 5.4.4.2 Chromium(III)

Other than a slight effect at 0.1 mg $l^{-1}$, there was little difference between the effect of chromium(III) on *Tisbe* at 20 or 30‰ salinity, see table 37. There was, however, some suggestion that the actual level of dissolved chromium(III) was lower in the test systems, as noted by the presence of particulates. This was not noted in either the measurement, or observation, of the excess solutions, held in the original glass volumetric flasks used for preparing the solutions.

The cause of these particulates is uncertain, but must relate to the test system, including the *Tisbe*, as no particulates were noted in the excess solutions. Similarly, the absence of particulates in the chromium(VI) exposure cells, suggests that the source was not *Tisbe* alone. The remaining possibilities are that the particulates were due to either the exposure cells, or *Tisbe*, exuding a chemical that interacted with chromium(III) at these higher concentrations, and formed a precipitate.

### 5.4.4.3 Chromium(VI)

As noted above, section 5.4.4.2, there were no particulates present in the exposure of *Tisbe* to chromium(VI). Chromium(VI) was much less toxic than chromium(III) at both 20 and 30‰ salinity. This is

similar to the results noted in the previous studies on sheepshead minnow and shrimp. It is worth noting that in both those studies, chromium(VI) continued to increase its toxicity with time, while that of chromium(III) did not. This is something that should be investigated with *Tisbe*.

Finally it should be noted that in these exposures, after 24 and 48 hours, no transformation of chromium(III) or (VI) to the other oxidation state had occurred.

### 5.4.4.4 Effect of EDTA

Although the analytical measurements indicated that chromium(III) was present at approximately the anticipated concentrations, the toxic response, *i.e.* the effect on *Tisbe*, was considerably lower when EDTA was present. This was noted at both salinities, although it was noticeable that at 30‰ salinity there was some reduction of this detoxification effect. The most probable explanation of this is the increase in "free" chromium(III), *i.e.* uncomplexed with EDTA. By calculation using MINEQL, there is 18 % of the 0.4 mg $l^{-1}$ chromium(III) uncomplexed at 20‰ salinity, (0.07 mg $l^{-1}$), which increases to 25 % at 30‰ salinity, (0.5 mg $l^{-1}$). This change in percentage "free" chromium(III) is less noticeable at the higher test concentration of 2 mg $l^{-1}$, changing from 23 to 25 % with the increase in salinity from 20 to 30‰, but there was a noticeable increase in predicted concentration from 0.09 to 0.5 mg $l^{-1}$.

The opposite effect was noted with chromium(VI) which had a greater toxicity at the lower salinity. There was also a slight amelioration of this toxicity when the concentration of EDTA was increased. This suggests that the toxicant may be chromium(III) which at the higher EDTA concentration was increasingly complexed. However, the presence of chromium(III) was not confirmed by analysis.

That EDTA does not effect the toxicity of chromium(VI) is given further support by the work of Mazidji *et al.*, (108). They reported that the toxicity of chromium(VI) in a Microtox test, which was a phytobacterium, did not alter with increasing concentrations of EDTA.

The possibility that the increased toxicity is due to EDTA, is unlikely, as the fourfold increase in EDTA concentration does not lead to a similar increase in toxicity. Also it would be expected that this effect would be more noticeable in the lower salinity test, when less EDTA is complexed by calcium and magnesium.

### 5.4.4.5 Effect of NTA

The impact of NTA on the toxicity of chromium(III) was similar to that of EDTA but reduced. Thus the toxicity was reduced below that reported for chromium(III) earlier, and was greater than that noted above when EDTA was present. This would be expected on the basis of the equilibrium constants for the respective complexes. The

NTA log (equilibrium constant) for chromium is greater than 10, while that of EDTA is 23.4, (109). However, MINEQL was used to compare the effect of concentration and salinity on the extent to which chromium(III) would have been bound by NTA, and predicted no complexation by NTA of chromium(III). This is in marked contrast to the situation noted above, section 5.4.4.2, and would suggest that either the detoxification mechanism is different from that being suggested, or that the effects are more subtle than is capable of being determined by either MINEQL or the analytical procedures being used in these experiments.

Within the limits of experimental error there was no impact of salinity on the toxicity of chromium(VI) when in the presence of NTA. The toxicity although reduced compared with EDTA was still enhanced over that in the absence of an organic ligand. In the absence of analytical confirmation it is difficult to ascribe this increase to chromium(III), formed from chromium(VI).

### 5.4.4.6 Effect of citric acid

There appeared to be a much reduced toxicity of chromium(III) at 20 and 30‰ salinity, which was partially mirrored by the analytical results. Thus at 20‰ salinity the analytically determined concentration of chromium(III) was reduced, but not at 30‰ salinity. It has been shown (25) that the addition of citric acid does lead to a

reduced electro-analytical response by both chromium(III) and (VI), and on that basis the chromium determined was referred to as reactive. However, the extent to which this reduction in the electrochemical response might be representative of bioavailable chromium, was not supported by this work, table 39, in which the concentration of chromium(III) as measured by the electro-analytical method was not very different when measured in the presence of EDTA, NTA or citric acid. Although there was a reduction in the concentration of chromium(VI), this was compensated for by the increased concentration of chromium(III).

This effect of the citrate ion to reduce toxicity has not been noted previously and this would suggest an area for future research into the amelioration of chromium(III) toxicity.

The effect of citrate on chromium(VI), however, was the reverse of that noted for chromium(III). Thus the toxicity of the citrate-chromium(VI) system was increased over that of chromium(VI) alone, particularly at the lower concentration of citrate and at 20‰ salinity. This lends weight to the suggestion that the toxicity was due to the formation of chromium(III), which then exerted its full toxicity, due to the removal of citrate by chromium(VI).

## 5.5 SUMMARY

It is clear from the work reported above that there still

remains a considerable number of questions about the toxicity of chromium(III) and (VI). The short term immediate toxicity of chromium(III) was unexpected, and the mode of action needs to be more fully explored. It may be a surface physical effect, which will have no longer term effect, and which would be affected by the form in which chromium(III) were present in the system being investigated. In favour of this is that fact that increased complexation of chromium(III) does lead to a reduced toxic response.

However, the analytical responses were not always in line with the toxic responses, particularly as exhibited by *Tisbe*. There is then a need to further refine the analytical methodology, and to explore further the impact of chromium(III) on this animal, *Tisbe*, in particular.

The toxicity of chromium(VI) had been thought to be due to its transformation to chromium(III). However, whether this necessarily occurs inside the animal is not certain. The data with the sheepshead minnow does suggest a gradual move into the body, followed by an increasingly toxic burden. This could be examined with the use of either a radio-isotope, *e.g.* $^{51}Cr$, or the mass isotope $^{53}Cr$, with analysis using an Inductively Coupled Plasma - Mass Spectrometer. However, this latter experiment would be very challenging to carry out in seawater, due to isotopic interferences from the formation of $^{37}ClO^{+}$ in the plasma system.

It is possible that the reduction of chromium(VI) to (III) is the mode of toxicity with *Tisbe*, however, it is less clear that it necessarily takes place in the organism. Thus in the presence of citric acid there was some formation of chromium(III) possibly resulting in the toxic response noted in that study.

It would appear on the basis of these experiments, with the knowledge of the analytical methodology available, that it is not yet possible to predict the biological impact of chromium in an estuarine environment. Neither is it possible to replace animal experiments to determine chromium toxicity, by predictions based on MINEQL, or anlalysis, alone, or in combination.

# CHAPTER 6.....CONCLUSIONS

## 6.1 INTRODUCTION

Chromium is widely used either in the chromium(III) or (VI) oxidation state. Although the majority of uses require the chromium(VI) ion, many of these uses have very little potential for the environmental release of chromium. Of those uses that do lead to the environmental release of chromium, including leather tanning, pigment manufacture and metal plating works, either oxidation state may be involved.

The importance of these discharges, and the respective oxidation state of chromium, arises from the different chemistry of the two forms. These differences affect both the behaviour in the environment and the ecotoxicity of chromium(III) and (VI).

Chromium(III) tends to form poorly soluble complexes, and adsorbs strongly to particulates at environmentally relevant pHs. In well oxygenated waters at about pH 8, chromium(III) will transform to chromium (VI), although this may not be sufficiently rapid to be of interest in an examination of its environmental behaviour. In reviewing reactions with $MnO_2$ bound to particulate matter, however, it appeared that such processes would occur at sufficient speed as to be important in the environment.

In contrast, chromium(VI) tended to be considered as more mobile, being less liable to adsorb to particles at pH 7 - 8, and forming very soluble chromate ions. In this form, however, chromium(VI) is a strong oxidising agent, and subsequently reacts with a range of organic ligands including fulvic acids. Furthermore it will also react with ferrous or sulphide particulate matter, underlining the importance of particle mediated transformation processes for both oxidation states.

In investigating the environmental levels of chromium, a range of analytical methods have been used. Few of the studies were able to combine speciation determinations, with a full analysis of the environmental matrix, including pH, dissolved oxygen or salinity. Furthermore very few studies were identified as having investigated the levels of chromium(III) and (VI) in estuaries.

The ecotoxicity of chromium(III) and (VI) presents a confusing picture, with chromium(III) tending to be more toxic in short term tests. Of the studies reviewed there was a suggestion that chromium(VI) became the more toxic with increasing time. The data also suggested that chromium(III) was less toxic in seawater compared with freshwater. However, these studies had not been carried out with supporting speciation determination of chromium(III) or (VI), hence the reason for these findings was uncertain.

The analytical methodology used to determine chromium(III) or (VI) has tended to attempt the determination of only one of the oxidation states, combined with an estimation of the total chromium. Chromatographic techniques have begun to show some potential for the simultaneous determination of chromium(III) and (VI), but at the time of review, most of the methods suffered from poor sensitivities or interferences. This was general for many of the differing techniques reviewed. A further criticism was that there had been no attempt to relate the measured concentration of chromium(III) or (VI) with the bio-available fraction.

In this programme of work, the aim was to investigate methods of analysis based on electrochemistry, which it was suggested held the best potential to overcome all of the above objections. This is due to the inherent sensitivity of such methods, allied with the ease with which operating parameters and organic ligands may be added to alter the analytical signals. A further advantage of such systems is that pH may also be easily altered within the electrochemical cell, leading to the potential to investigate other effects in-situ.

## 6.2 MODELLING SUMMARY

In an attempt to aid understanding of the experimental data, a computer programme, MINEQL, capable of calculating equilibrium concentrations was used. Such programmes

using thermodynamic data are capable of helping, by predicting metal forms, and their concentrations, under a set of specified conditions. This then enables a range of different conditions, either environmental or artificial, as in an electrochemical cell, to be explored.

The disadvantages of using an equilibrium model, however, mean that it is not possible to explore kinetic effects. Thus, for example, in model solutions containing chromium(III) and (VI), the program predicted all the chromium would change to chromium(VI). Other concerns arising from the use of such programmes are due to the lack of data relating to thermodynamic equilibrium constants and the need to transform such values, obtained from measurements at one ionic strength, to a different ionic strength in the sample.

Provided these limitations are understood, such models are capable of helping investigate the possible distribution of various chemical forms in a wide range of matrices.

Consequently, the model was useful in predicting that the electrochemical method to be investigated, was likely to require an altered pH for maximum sensitivity over that originally reported. The shift was not as drastic in concentration as had been suggested elsewhere, (25), and this was investigated further.

The principal difference appeared to be related to the

effect of magnesium as the major competing cation for DTPA. Thus in a MINEQL calculation based only on the presence of typical levels of magnesium in river and seawater, with 2.5 mM diethylenetriaminepentaacetic acid, DTPA, the free concentration of $DTPAH_2$ was calculated to be 25 μM, a similar value to that reported. However, with the addition of chloride to the system, this calculated concentration increased to over 250 μM, due to complexation of magnesium. Clearly this would continue to increase as other anions were added which would also complex magnesium.

The model also predicted that at equilibrium, chromium(III) would be mostly present as $Cr(OH)_4^-$ in seawater, and that there would be a minimal impact of adsorption and organic ligands. This finding was, however, based on limited data, and there does appear to be a need for a fuller exploration of the impact of complexing material, on chromium(III). Similarly, although the model was unable to help in understanding the behaviour of chromium(VI), the effect of naturally occurring ligands on the possible transformation between the two oxidation states needs continued investigation.

## 6.3 ELECTROCHEMICAL SUMMARY

Initial electrochemical investigations using cyclic voltammetry, CV, were designed to understand the behaviour and reactions of chromium(III) and (VI). The complexing

ligand used was diethylenetriaminepentaacetic acid, DTPA, and the solutions were buffered with sodium acetate at pH 6.2. These early experiments demonstrated that 3 peaks were present when the CV scan was extended to -1.7 V. It was suggested that these represented the cathodic reactions, chromium(III) -> (II) and (II) -> (0), and the anodic reaction, chromium(II) -> (III). When investigated by itself the redox reactions of chromium(III) and (II), were reversible, (25, 71). However, in this study, with the extension of the CV scan to -1.7 V, the reactions behaved in an irreversible manner. This was probably due to the irreversible nature of chromium(0), formed at -1.6 V.

One finding, noticed by others, (25, 73), was the decline in the chromium(III) response with time. By determination of total chromium, this was shown not to be due to the loss of chromium(III) from solution. The possibility that it was due to the complexation of chromium(III) by DTPA was demonstrated, as such complexation led to a reduced response. A suggested area for future work would be to investigate the relationship between the concentration of DTPA versus the chromium response, and the rate of reduction in the electrochemical response with time.

The effect of nitrate ion in catalysing the chromium(III) -> (II) electrochemical response was confirmed. The possibility that chromium(VI) operated in a similar way, provided the CV scan rate was 100 mV $sec^{-1}$

or lower, was explored. The effect was not as large as that obtained with nitrate ions, and was lost at higher CV scan rates.

One object of the work was to be able to determine chromium(III) in the presence of chromium(VI). This led to several experiments to remove chromium(VI) by the addition of lead. This had the desired effect, but also produced an enhanced response in the chromium(III) -> (II) peak. Examination showed that this increased response occurred at the preconcentration stage, suggesting that it was due to lead forming an amalgam with the mercury, at the plating potential, which subsequently took part in a ligand exchange reaction with chromium(III). This could be confirmed by the use of a media exchange system, such as that described in section 3.7.3. Thus putting lead in the complexation but not the stripping reagent, should give the increased chromium(III) -> (II) peak.

Unfortunately when developing the analytical method based on this response, using square wave voltammetry, a large interference peak appeared. The use of simplex optimisation to reduce the impact of this interference showed that the signal to noise ratio could be improved, in this study, by having a frequency of 100 ± 20 Hz, and a scan rate of 8 ± 2 mV. However, the lead induced interference could not be eliminated, and further investigation demonstrated that the response was possibly due to a chemical reaction involving iron, present in the

mercury drop.

These findings meant that a separate determination of chromium(III) from chromium(VI) was not possible. Subsequently a media exchange system was set up to determine chromium(VI) and total chromium(VI).

The use of media exchange to investigate the responses obtained at environmentally relevant concentrations was successful for two reasons. Firstly it was only through the use of an alumina pre-column that chromium(III) and chromium(VI) were separated and a speciation scheme obtained. Secondly, one of its major benefits was that it was possible to separate the stripping and adsorption stages, and investigate the impact of salinity and pH on these two steps. One of the more surprising findings was the extremely sensitive response obtained when pH during the stripping step was lowered. While this was expected for the adsorption step, due to the altering complexation of DTPA, see section 6.1 above, there appeared to be no reason for this to happen at the stripping stage. It is recommended that a full exploration of these factors be made with the media exchange system. By further lowering the pH it might show whether the electrochemical response was being catalysed by the hydrogen ion. The other alternative would be that the nitrate oxidation of chromium(II) is pH sensitive, and is faster at lower pH than higher.

In investigating the effect of using Britton and Robinson buffer in the presence of chromium(VI) it was discovered that a large peak, proportional to chromium concentration was obtained. The contributing agent was narrowed down to citric acid, but the probable mechanism, that of reduction of the chromium(VI) within the cell, was not confirmed. It is recommended that this effect be more fully investigated. The development of a media exchange system should aid this, as it would again be possible to separate the adsorption step from the stripping step, and thus identify which of these two plays the more important part. Clearly, if citric acid is reducing chromium(VI) to nascent chromium(III) which is highly reactive, separating the stripping stage and removing the citric acid from the stripping reagent, will have no effect on the response. If, however, citric acid is catalysing the response during the stripping stage, then citric acid in the pre-concentration solution would have no effect on the response, whereas if it were in the stripping reagent there would be an increased, and altered response.

This response of chromium(VI) to citric acid was developed into an analytical technique for the determination of chromium(VI). The method is of limited use at this stage, being prone to complexation of citric acid, *e.g.* in seawater. The method does have potential for use in potable waters, or similar matrices, for the selective and sensitive determination of chromium(VI) with the minimum of sample handling..

## 6.4 ENVIRONMENTAL SUMMARY

The attempt to explore fully an estuary and how chromium speciation altered within it, with respect to the differing inputs, and changing characteristics as the river flows out to sea, and mixes with the seawater, was unfortunately unsuccessful. However, it did indicate the presence of an interferent within the estuary which needs to be more fully explored. This interferent could not be identified, and the area of its release into the Tees was similarly unidentified. It should, however, given the type of effect it had on the electrochemical cell in use in this programme be comparatively straightforward to find. The possible use of an external reference electrode was demonstrated as being one alternative, which would minimise the interference, thus allowing further work to take place.

The importance of measuring a wide range of other determinands was demonstrated. Thus the large oxygen sag in the Tees estuary would undoubtedly lead to changes in the oxidation states of chromium.

The method used is capable of being used on board ship, as demonstrated within the programme. It is recommended that it be used to investigate other areas to explore the fate of chromium, and perhaps determine whether the contaminant obtained on the Tees is an isolated incident, or one of a more general nature.

## 6.5 ECOTOXICOLOGICAL SUMMARY

The object of this series of experiments was to understand more fully the toxicity of chromium(III) and (VI) in estuarine waters. The species chosen, and the salinities at which they were tested, were more representative of lower estuarine conditions, *i.e.* 20 ‰ salinity and higher. It is recommended that other species, more representative of the upper estuary should be subjected to a similar series of tests. This would aid in the understanding of the toxicity of chromium(III) and (VI) in an estuary, and could aid further understanding of the data presented here.

In examining the toxic response of chromium it was confirmed that chromium(III) was more toxic than chromium(VI) in short term studies. However, as the time period was extended, chromium(VI) was either more toxic or likely to become more toxic. The reasons for this are not clear, but speciation analyses suggest that the cause is not due to conversion from one oxidation state to the other, in the test system. Chromium(III) appears to have a short term effect, that does not appear to cause a longer term problem to the organisms, in the tests carried out in these studies. Chromium(VI) on the other hand possibly moves into the organism's body, where it then exerts a toxic response either through the effect of conversion to chromium(III) or as chromium(VI).

Investigating the possible site of the toxic effects of the two chromium states, would be very difficult. Initial experiments might look at selected organs of the animals under test, for total chromium. These studies could be followed by investigations using high performance liquid chromatography linked to an ICP-OES or ICP-MS to allow the separation of proteins or other organics associated with chromium. In this way the target organs and associated organics might be identified.

The other effect noted, was that chromium(III) tended to be more toxic at lower salinities. While this was only noted for *Crangon crangon*, the data obtained in the *Tisbe tests* was possibly confounded by the formation of some precipitate in the test system. This increased toxicity at lower salinity of chromium(III) needs further investigation to confirm the trend. A possible explanation would be that the free chromium(III) ion is increasingly complexed by chloride ions at high salinities. Thus the available ions for exerting a toxic response are reduced.

The increasing complexation of chromium(III), might also explain the reduced toxicity of chromium(III) when organic ligands are present. The argument is further strengthened by the increase in the toxicity of chromium(III) to *Tisbe* at increased salinity in the presence of organic ligands. Since at the higher salinity the organic ligand is liable to be increasingly complexed by calcium and magnesium

ions. This was predicted by MINEQL for EDTA, although not for the other ligands.

The effect of the same organic ligands on the toxicity of chromium(VI) was unexpected. The analysis of the solutions suggested that there was some conversion to chromium(III) when citric acid was present. However, this did not occur, or was not detected, in the presence of EDTA or NTA. This needs further investigation, perhaps by measuring the rate of conversion of chromium(VI) to (III) in the presence of these ligands in differing salinities.

Finally it was not possible to properly discriminate between bioavailable chromium and electro-active chromium, as determined with the system described in this study. This should be further explored, perhaps by altering the ligand used in the complexation of chromium prior to the electrochemical determinations.

Another way of aligning the analytical signal to that of the biological response, would be to replace the mercury drop with a solid glassy carbon electrode coated with mercury, (75), *i.e.* a thin mercury film electrode, and covering it with a nafion film. Alternatively, a chemically modified solid phase extractant, could replace the alumina in the media exchange system. This approach has been shown to relate the measured concentration of copper, to the bioavailable fraction for at least one species of algae, (76). However, a study of this type

would require considerable time, and a favourable juxtaposition of an organism, its toxic response, and the measured level of extracted chromium.

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