ELECTROCHEMICAL STUDIES OF GOLD BIOACCUMULATION BY YEAST CELL WALL COMPONENTS

THESIS

Submitted in Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

of

RHODES UNIVERSITY

by

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JANUARY 1999

ACKNOWLEDGEMENTS

My sincerest thanks and appreciation to Professor Nyokong for the outstanding supervision of my Masters studies. Her high standards, dedication, enthusiasm and attitude towards research are an excellent example for her peers and students alike. I am indeed privileged to be associated with the "Nyokong-Machine".

Professor Duncan, who has co-supervised my Masters studies, I thank for his input in initiating this research and his correspondence with Goldfields Mining Company on my behalf. Professor Duncan's interest in applying research to industry has made for some exciting challenges, which will continue to bridge the gap between biological and applied sciences. I am immensely grateful to both supervisors for their collaborative efforts during the course of my studies.

I acknowledge the following organisations for their financial assistance:

The Foundation for Research and Development in South Africa
Rhodes University

My appreciation is extended to the following people:

My parents, Brian and Emelia, brother David and grandparents, Cecil and Murial Lack and Verita Allner for their love, guidance, patience, support, many sacrifices and financial assistance through all my years of study to ensure I received the best possible education.

The staff and postgraduate students of the chemistry department for their support and encouragement. Margie Kent and Benita Tarr for their kindness. Andre Adriaan for making the specialised glassware required in the experimental work. Andre Daubinet and Professor Kaye for their help with computer modelling of the compounds. Mike Datt and Rosa Klein for their friendship, help in proof-reading this thesis and assistance in the NMR studies. My research group for their kindness, friendship, sharing of ideas and support.

Dr Cross and Shirley Pinchuck for assistance in the electron microscopy studies.
Dr Leslie for her time and effort in the NMR studies. Dr Watkins for his advice in the IR spectroscopy.

To my friends who have encouraged, supported and beared with me through this thesis; my heartfelt thanks. Kim Wilkins, Mez Jiwaji and Ashleigh Wilson for their help and advice in poster preparation for all the conferences attended. The hall warden, warden and students of Phelps House for their friendship and encouragement of my work. I am indebted to Ruth Nathanson and Steven Robinson for their help with the thesis layout, continued friendship and support.

ABSTRACT

Gold, amongst other group 11 metals, was almost certainly one of the first three metals known to man. In addition to the economic importance of the metal, gold has a wide variety of applications in the medical, electrocatalytical and micro-electronics fields. However, the determination of gold ions in solution, with accuracy, precision, sensitivity and selectivity is still an interesting and much debated topic in analytical chemistry.

A system whereby gold ions have been successfully detected employing an electrochemical technique, known as stripping voltammetry, has been developed. The electrochemical method was chosen over other available techniques for the sensitivity, particularly at low concentrations, and selectivity properties; notably in the presence of other metal ions. Under acidic conditions, the electrochemical technique was applied and the presence of gold(III), at a concentration of 2.53×10^{-5} mol dm^{-3} in a mine waste water sample, was detected.

Biomass, in particular yeast and algal types, have been successfully employed in extracting low concentrations of gold ions from industrial effluents. The manipulation of the biological facility for mineral interaction, biohydrometallurgy, may yield numerous potential new technologies. South Africa in particular would benefit from this area of research, since the country is a major ore and metal refining country and if the output and the efficiency of the mines could be improved, even by a small percentage, the financial rewards would be vast.

In this study, the application of adsorptive cathodic stripping voltammetry (AdCSV) of gold(III) in the presence of various *Saccharomyces cerevisiae* cell wall components, was investigated to determine which, if any, were involved specifically in the chemical binding of the gold ions. The chitin and mannan extracts showed the most promise with detection limits of 1.10×10^{-6} mol dm^{-3} and 9×10^{-9} mol dm^{-3}, respectively; employing the AdCSV technique. A modification of the stripping voltammetry technique, Osteryoung square wave

stripping voltammetry (OSWSV), provided the lowest detection limit, for gold(III) in the presence of mannan, of 1.70×10^{-11} mol dm^{-3}; utilising a modified carbon paste electrode.

The detection of gold(III) has been shown to be dependent on the type of electrode employed, the electrolyte solution and the presence of interfering agents. The effect of copper(II) and silver(I) on the detection of the gold(III) in solution was investigated; whilst the silver(I) has shown no detrimental effects on gold(III) detection systems, copper(II) has indicated the possibility of forming an inter-metallic compound with the gold(III). However, mannan has shown to selectively and preferentially bind the gold(III) in the presence of a ten-fold excess of copper(II).

Nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy, as well as computer modelling techniques were employed to further investigate the mannan-gold(III) interaction and proposed complex formed. The NMR, IR and computer modelling data are in agreement with the electrochemical data on proposing a mannan-gold(III) complex. The co-ordination site was established to be in the vicinity of the H-1 and H-2 protons and the gold(III) adopts a square-planar geometry upon co-ordination.

The benefits of the research are useful from a biological perspective (*i.e.* as more is known about the binding sites, microbiologists/biochemists may work on the optimisation of parameters for these sites or work could be furthered into the enhanced expression of the sites) and an industrial one. In addition to the two major benefits, an improved understanding of gold and its chemistry would be achieved, which is advantageous for other fields of research as well.

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List of Abbreviations

AdCSV	Adsorptive Cathodic Stripping Voltammetry
ASV	Anodic Stripping Voltammetry
COSY	^{1}H-^{1}H homonuclear shift correlated spectroscopy
CPE	Carbon Paste Electrode
DPV	Differential Pulse Voltammetry
FTIR	Fourier Transformed Infrared
GCE	Glassy Carbon Electrode
HOD	Hydrogen Oxygen Deuterium
ICP-MS	Inductively Coupled Plasma- Mass Spectroscopy
IR	Infrared spectroscopy
OSWSV	Osteryoung Square Wave Stripping Voltammetry
MCPE	Modified Carbon Paste Electrode
NMR	Nuclear Magnetic Resonance spectroscopy
RDP	Reconstruction and Development Program
SEM	Scanning Electron Microscope

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INTRODUCTION

1.1 Metals as pollutants to a valuable resource: water

Water is the most important, although often underrated, natural resource on earth. Water supply is related to the basic quality of human life and the aim of the Reconstruction and Development Program (R.D.P) in South Africa is to supply every person in this country with 25 *l* of potable water (water fit for human consumption) per day within a radius of 200 m of their residence.[1]

The availability and quality of water are of paramount importance for socio-economic growth and development in South Africa.[2] At present, less than 10 % of the total water available to the South African population is used in industrial processes, whereas in the more developed countries industry presently accounts for more than 40 % of the water usage. Industrial development will result in increased demands on this valuable resource, a possible decline of water quality may occur and more stringent pollution control regulations will be enforced.[3]

South Africa experiences inter-annual variability with drought and wet years occurring regularly and beyond the year 2020 the predicted demand for potable water will exceed the available supplies.[2] With such a high demand being placed on limited quantities of water, preventing or at least limiting its tainting by pollutants is essential.

One of the most commonly encountered group of water pollutants are metals. Metal contamination of the lowest strata of the food chain will have cumulative effects throughout, an example of this fact is the 1950's Minimata tragedy in Japan.[4, 5] The term 'metals' is generally used to describe the highly toxic transition metals such as cadmium, lead and mercury, those toxic at high concentrations such as copper and cobalt, the precious metals including silver and gold and the radionuclides.[6 - 8]

Sources of metal pollution include the nuclear power industry, defence and fuel reprocessing operations, surface finishing and electroplating processes, mining operations, smelting processes and the textile industries.[9]

The mining industry, however, remains the single most important industry in South Africa. Despite the fact that mining activities constitute a minor usage of the national water supplies (less than 3 % ; the gold mining industry consumes a slightly higher percentage of freshwater, but recycles over 80 % back into the operation), the industry is a significant contributor to water pollution and hence will play an important role in the overall scheme of water availability and quality.[10]

The proposed maximum limit of gold permitted in drinking water is 5 $\mu g\ l^{-1}$, which is much lower than for lead (100 $\mu g\ l^{-1}$); a toxic heavy metal.[11] Toxicity is not the only disadvantage to the discharging of metals into the environment. Another salient point is that metals are expensive to locate, mine and refine. The slow natural formation of metal deposition results in metals being considered as a non-renewable resource and should thus be managed with care.[12]

Research into the possibility of metal reclamation from discharged waste or minimising losses during metal processing presents the opportunity to produce cheaper final products, with a higher profit margin. For South Africa in particular, the above area of research is one of singular importance, since this country is a major metal ore mining and refining nation, and the country contains a substantial percentage of the world's known valuable metal resources.[13]

South Africa is one of the few countries in the world facing such an immediate water shortage and a dependence on a non-renewable resource (metals). Conservation effective measures against pollution and research into the recycling of the resources have been identified as the areas of importance by the relevant authorities for preservation.[14]

1.2 Bioremediation of metal-laden effluents:

1.2.1 A general overview:

Bioremediation refers to a biotechnological process whereby metal ions are removed from waste waters.[15] The link between metal ions and micro-organisms is not a new one. Microbial fossils have been associated with high metal concentrations in various geological formations. Micro-organisms have long since been known to play important roles in the solubilisation and laying down of minerals in the natural environment. The strata of gold in the South African Vaal Reef, for example, may be the result of a prehistoric deposit of gold ions chelated to biological material in the sedimentary level of an ancient river bed.[16, 17]

Moreover, during the last 200 years following the start of the industrial era, the redistribution of many metals has occurred forcing elevated levels into the environment of the microbes and other living organisms. In order to survive, the organisms have had to adapt to their environment necessitating higher metal tolerances and metal resistance levels for survival. The many varied reports of certain plant species, filamentous fungi, yeast, algae and bacteria all possessing metal remediation properties are thus not unexpected.[18]

Most biological matter may be viewed as potential metal biosorbents; that is possessing the ability to bind and concentrate various metals allowing for the eventual recovery of these metals and their re-incorporation into industrial processes. The term "biosorption" is now frequently used to encompass uptake by biomass via an energy-independent, physico-chemical based interaction between metal ions and the surface of the species.[19]

Bioremediation is a growing field as effluent and water treatment become ever more important to industry. Interest in the utilisation of microbes in bioremediation has been stimulated by the fact that micro-organisms are found in almost every imaginable environment and are estimated to have their total cell biomass, on earth, of twenty-five

times the total mass of animal life. Micro-organisms also offer a range of advantages including a wide range of biochemical activity, rapid growth rate and relatively simple experimental requirements in comparison to higher order life forms such as plants.[20]

The recovery of metals from solution would be beneficial since the metal recovered may be subsequently desorbed from the biomass and recovered for re-use. The release of potentially toxic metals into the environment would also be restricted. Conventional existing metal removal technologies include metal ion exchange, evaporative recovery, electrochemical treatment and chemical reduction procedures. Bioremediation offers an alternative to these procedures.

The ability to recycle the biomass and the use of waste biomass from industry as biosorbents, would increase the economic competitiveness of the bioremediation technology. Any disadvantages experienced by bioremediation methods, compared with existing technologies, could be compensated for by the high metal binding capacity and selectivity of the micro-organism for certain metals. Waste yeast biomass, for example, represents a good source of biosorbent; because it is cheap, easily recovered at the end of fermentation and is produced in large quantities.[21]

The method of biomass metal recovery is organism and species dependent. However, the metal uptake procedures occur via several known pathways, such as: (1) biosorption of the metal ion onto microbial cell surface, (2) intracellular uptake of metals, or (3) their precipitation through compound formation with microbially-produced ligands. The latter two processes have found some use in the water purification and mining fields, but suffer from the disadvantage that living organisms are required for these processes.[22]

The utilisation of surface-bound recovery processes, however, has a number of advantages over the latter two methods. The surface-bound process functions equally well, or even more effectively with a suspension of dead cells, which can suffer de-naturation of the cell

wall and allow free access to cell wall binding sites; additionally nutrient material costs required by viable cells would be averted.[23]

Until recently, activated carbon has been employed in the adsorption and recovery of gold from aqueous solutions (the carbon-in-pulp process). However, the manufacture and regeneration of this material provided several constraints and represent a major portion of the operating costs of a conventional sorption process. The use of biosorbents from natural sources, which may possess a superior sequestering power compared to commercially used ion exchange resins or activated carbon, would represent a substantially cheaper alternative recovery process and would enhance the effectiveness and feasibility of metal recovery method.[24]

1.2.2 Structure of Saccharomyces cerevisiae: a possible biosorbent

Yeast microbes are the most important and extensively used micro-organisms in industry today. Various strains of yeast have found applications in the brewing, baking, animal feed, food supplement and distilled beverage industries to name a few.[25] The most commonly encountered strain is *S. cerevisiae*. Although essentially a brewing yeast, the use of *S. cerevisiae* in the bioremediation of metal contaminated waters has gained considerable interest of late[21]

S. cerevisiae has been linked to the bioremediation of several heavy metals, including cobalt(II), copper(II) and cadmium(II), as well as the removal of precious metals, such as silver, from various waste water sources. The scanning electron micrograph in Figure 1.1 shows the *S. cerevisiae* cell morphology.

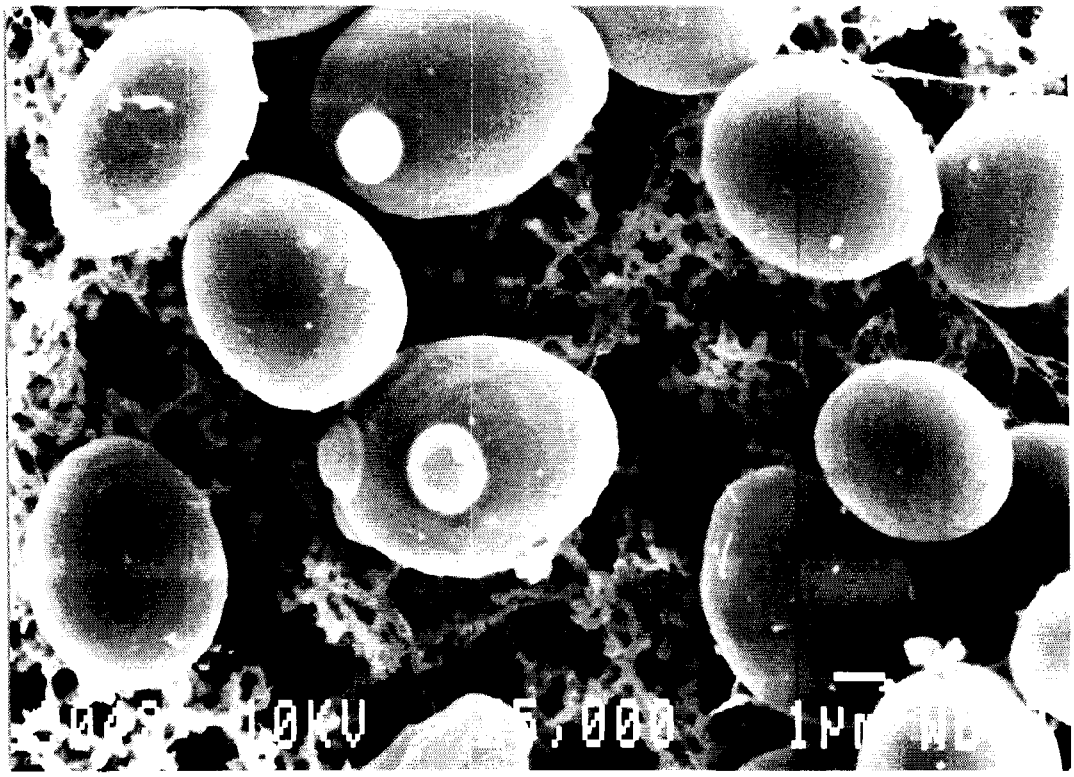

Figure 1.1: Scanning electron micrograph of *Saccharomyces cerevisiae* cells.

Fungal metal uptake is essentially a biphasic process consisting of metabolism-independent and metabolism-dependent steps. The initial biosorption step is rapid, typically only a few minutes in duration and is independent of temperature, metabolic energy, the presence of a metabolisable energy source and metabolic inhibitors. The initial binding phase has been attributed to microbial cell wall interactions, although in some cases extra cellular polymers may be responsible.

Biosorption is exclusively responsible for metal accumulation by non-viable biomass owing to the absence of metabolic activity necessary for intracellular metal accumulation. Metal to biomass ratios below 100 nmol g^{-1} have indicated that metal accumulation then depends almost entirely on the biosorption of the metal ions to the cell wall. The ranging affinities of

microbial cell walls for specific metal ions may be attributed to differences in cell wall composition and chemistry.[26]

The wall fraction of the yeast cell, *S. cerevisiae*, has a layered structure and is 70 ± 10 nm thick. The bilayered structure consists mainly of intermeshed polysaccharide microfibrils, separated into an inner amorphous net of microfibrils and an interwoven fibillar outer layer. Mannan constitutes 31 %, glucan 28.8 % and chitin and chitosan are each responsible for 1 % of the total dry mass of the cell.[13, 27] The structure of mannan is illustrated in Figure 1.2(a).

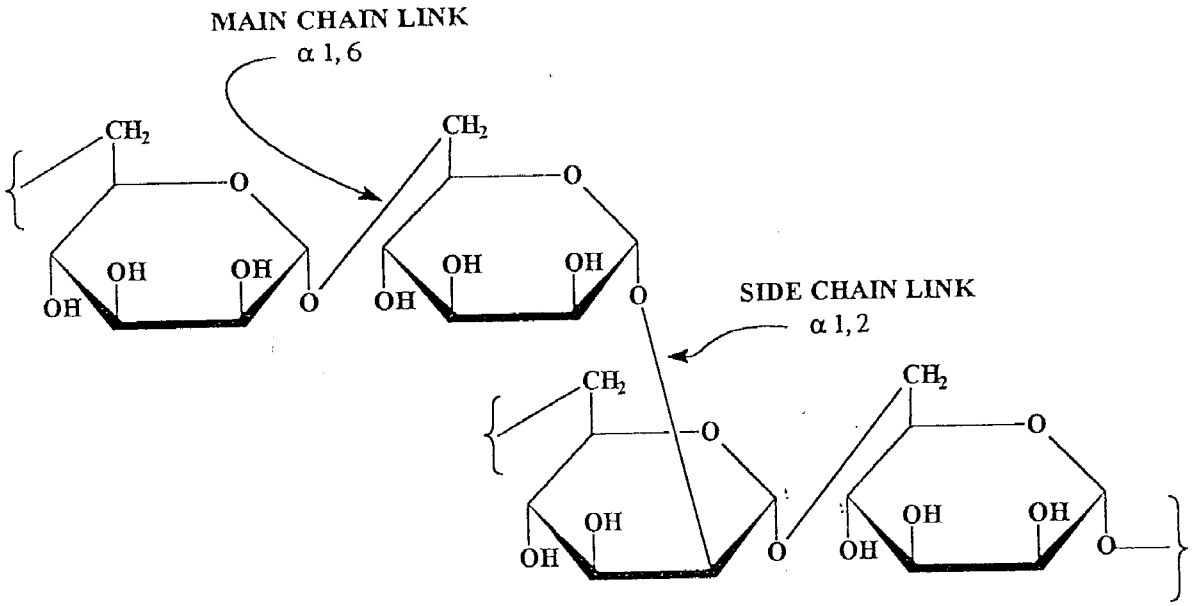

Figure 1.2(a): The structure of mannan; a *S. cerevisiae* cell wall polysaccharide.

In yeast, the mannan is found as a covalently linked protein-polysaccharide complex of 25 to 500 kDa, of which the protein usually only contributes 5 to 10 %. Mannan is a polymer of mannose monomers forming a main chain linked via α(1,6) bonds and side chains with

α(1,2) and α(1,3) bonded mannose residues, which branch from the main chain via α(1,2) links. The outer mannan-protein layer of the yeast cell has been shown to be more important than the inner glucan-chitin layer in the binding of certain metal cations.[27, 28]

Chitin is a polymer of the *N*-acetylglucosamine residues linked via β(1,4) glycosidic links and associated with a protein component too. The chitin is found as microfibrils in the inner layer of the cell wall in the glucan matrix. Chitosan is the deacetylated version of chitin and is located naturally in most fungal cells.[27] The structure of the chitin, and chitosan polymers are illustrated in Figures 1.2(b).

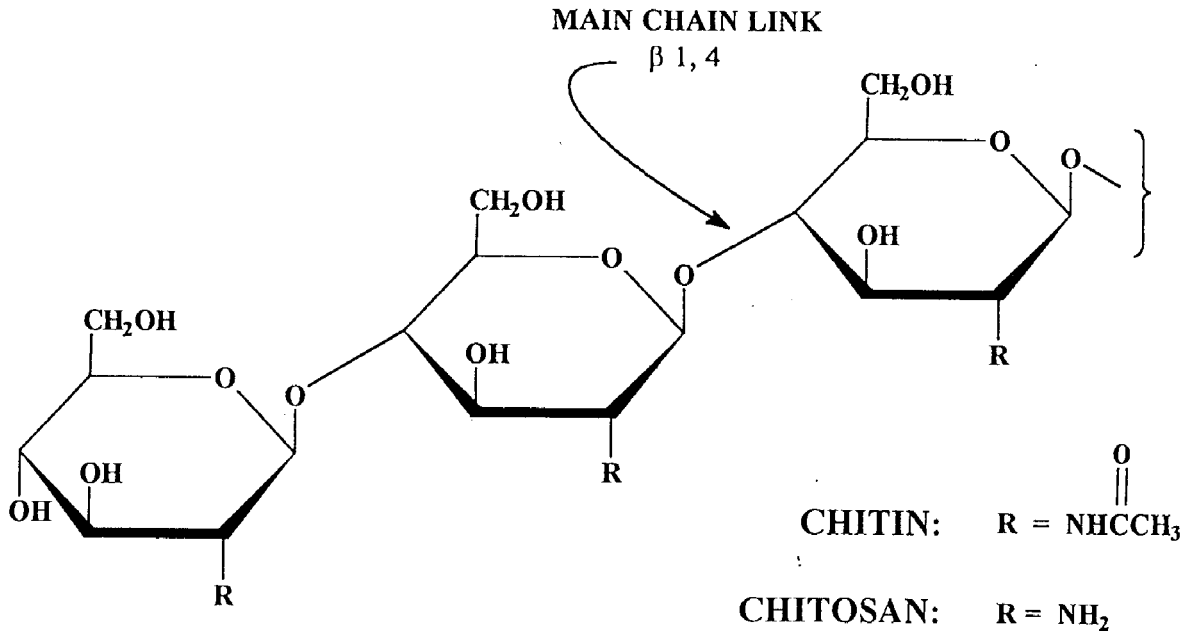

Figure 1.2(b): The central motif of chitin and chitosan.

The dissociation of the amine in solution provides heavy metal co-ordination sites via the lone pair of electrons, for the chitin extract, as seen in Equation (1.1).[13]

$$R\text{-}N^{+}\text{:}H + H_2O \leftrightarrow R\text{-}N\text{:} + H_3O^{+} \quad \text{..... Equation (1.1)}$$

Glucan, a polymer of β(1,3) linked glucose with β(1,6) branches, is primarily located on the cell membrane side of the cell wall. The glucan polysaccharide has not been considered previously as a primary candidate for heavy metal binding. However, as a result of the high percentage of glucan present in the yeast cell wall, an indirect binding metal cation binding system is proposed.[18, 27, 28] The structure of the glucan polysaccharide is depicted in Figure 1.2 (c).

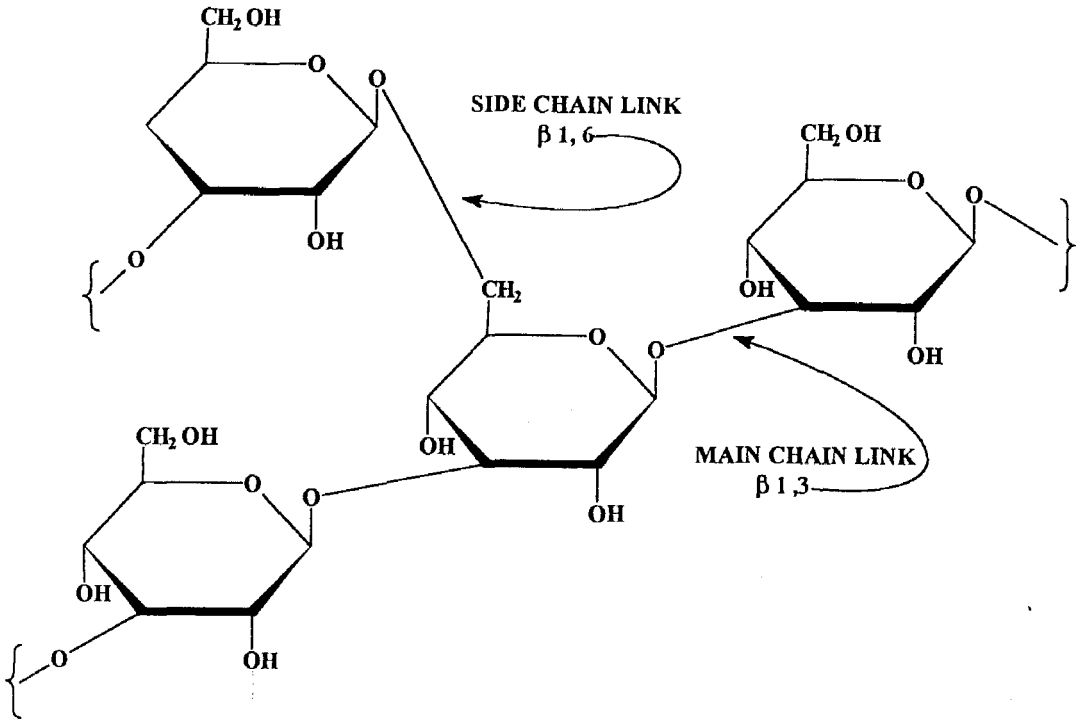

Figure 1.2(c): The structure of the glucan polysaccharide of *S. cerevisiae*.

In addition to the polysaccharide components, proteins and lipids occur within the wall. A small percentage of the cell wall may be comprised of inorganic ions such as calcium and magnesium. The isolated components of the cell wall have been shown to accumulate greater quantities of various cations than the intact cell wall, whilst the adsorptive capacity of the yeast cell wall for heavy metals is not determined by the protein component alone.

However, the structural organisation of the entire protein carbohydrate-complex and the degree of dissociation of the negatively charged functional groups has increased their

accessibility to metal-binding.[26] The metals concerned in these studies have chiefly been in the toxic heavy metal group. Trials on precious metals, such as silver, have recently been investigated, but to date the binding capacities of the various components, to the gold ions, have not been examined.[21, 26, 27]

1.3 The electrochemical perspective of metal interactions:

To date, electrochemical methods continue to be of importance in the environmental water analysis processes, particularly in the trace metal detection fields.[29]

1.3.1 The theory of electroanalysis:

Electroanalytical techniques are concerned with the interplay between electricity and chemistry, namely the measurements of electrical quantities, such as current, potential, and charge and their relationship to chemical parameters. In contrast to many chemical measurements, which involve homogenous bulk solutions, electrochemical processes take place at the electrode-solution interface. The two main types of electroanalytical measurements are potentiometric and potentiostatic. Potentiometry is a static (zero current) technique in which information about the sample composition is obtained from measurement of the potential established across a membrane.

Potentiostatic (controlled potential) techniques are based on dynamic situations (no zero current). Here the electrode potential is used to drive an electron-transfer reaction and the resultant current is measured. Any chemical species that is said to be electroactive, *i.e.* that can undergo oxidation or reduction, can be measured by potentiostatic techniques. Both types of electroanalytical techniques require at least two electrodes (conductors) and a contacting sample (electrolyte) solution, which constitute the electrochemical cell. The electrode surface thus acts as a junction between an ionic and an electronic conductor.[30]

One of the two electrodes will respond to the target analyte(s) and is thus termed the indicator (or working electrode). A suitable working electrode should provide high signal-to-noise characteristics, as well as a reproducible response. The second electrode, termed the reference electrode, is of constant potential (that is, independent of the properties of the solution) to which other potentials of the system may be referred to in terms of a potential difference, with respect to the chosen reference potential. A suitable reference electrode is required to have a potential that is stable with time and temperature changes, and which is not altered by small perturbations to the system (*i.e.* by the passage of a small current).[31]

The requirements imposed on the working electrode are as follows: electrochemical inertness over a broad potential interval; high hydrogen and oxygen evolution over-voltage; low residual current (absence of pores and pronounced roughness of the surface); low ohmic resistance; and the possibility of a sufficiently simple surface regeneration. All these factors should provide high accuracy, sensitivity and reproducibility of the results and low detection limits. Unfortunately there are no electrodes that satisfy all the requirements needed. The chosen working electrode should, however, strive to fulfil the majority of these requirements under the specified experimental conditions.[32]

Despite the adequacy of the two electrode system, a three electrode approach is preferred since a major portion of the cell resistance is compensated for by the presence of an auxillary electrode and a series of operational amplifiers and current loops. The auxillary electrode is the current-carrying electrode and is placed in solution to complete the current path. Current flow has now been removed from the reference electrode, which has been placed closer to the working electrode, causing a potential drop to be minimised and hence decrease the cell resistance.[30, 31, 33]

Since potentiostatic techniques were employed in this study a closer look at the theory of this group of techniques is required. The objective of controlled-potential electroanalytical experiments is to obtain a current response which is related to the concentration of the

target analyte. This objective is accomplished by monitoring the transfer of electron(s) during the redox process of the analyte:

$$O + ne^- <---> R \qquad \text{..... Equation (1.2)}$$

where O and R are the oxidised and reduced forms, respectively, of the redox couple. The above reaction will occur in a potential region that makes the electron transfer thermodynamically or kinetically favourable. For systems controlled by the laws of thermodynamics, the potential of the electrode can be used to establish the concentration of the electroactive species at the surface [$C_O(0,t)$ and $C_R(0,t)$] according to the Nernst Equation:

$$E = E^0 + \frac{2.303\,RT}{nF} \log \frac{C_O(0,t)}{C_R(0,t)} \qquad \text{..... Equation (1.3)}$$

where E^0 is the standard potential for the redox reaction. The R term is the universal gas constant ($8.314 JK^{-1}mol^{-1}$), T the temperature in Kelvin, n the number of electrons transferred in the reaction and F is the Faraday constant (96 487 coulombs per mole of electrons). The resulting current-potential plot, known as the voltammogram, is a display of current signal (vertical axis) vs. the applied potential (horizontal axis). The exact shape and magnitude of the response are governed by the process involved in the electrode reaction. The total current is a summation of the faradaic currents for the sample and blank solutions, as well as the non-faradaic charging background current. The pathway of the electrode reaction can be quite complicated and takes place in a sequence that involves several steps.

Simple reactions involve only mass transport of the electroactive species to the electrode surface, the electron transfer across the interface and the transport of the product back to the bulk solution. More complex reactions include additional chemical and surface processes which precede or follow the actual electron transfer. The net rate of the reaction,

and hence the measured current, may be limited either by mass transport of the reactant or by the rate of electron transfer. The more sluggish process will be the rate-determining step. A given reaction may be controlled by mass transport or electron transfer, which is usually determined by the type of compound being studied and by the various experimental conditions applied (chosen electrode material, operating potential, mode of transport, time-scale *etc.*).

Mass transport occurs via three different modes: (1) Diffusion - the spontaneous movement under the influence of concentration gradient, *i.e.* from regions of high concentrations to regions of lower concentrations of the sample, aimed at minimising concentration differences. (2) Convection- transport to the electrode by a gross physical movement; such fluid flow occurs by stirring or flowing the solution and by rotating or vibrating the electrode (*i.e.* forced convection) or because of density gradients (*i.e.* natural convection). (3) Migration- movement of charged particles along an electric field (*i.e.* the charge is carried through the solution by ions according to their transference number).[30, 31, 33, 34]

1.3.2 An overview of gold and associated redox potentials:

Gold is perhaps the most beautiful of the chemical elements and has been known, used and treasured by man since the earliest times. Despite the economic and monetary importance of the metal, gold has found a wide variety of other uses in the medical, electroanalytical and micro-electronic fields. The determination of gold ions, in solution, with accuracy, precision, sensitivity and selectivity remains a topic of interest in analytical chemistry today still.[35, 36]

The element gold, has an atomic number of 79 and is found in group 11 of the Periodic Table; along with platinum and silver. Naturally occurring as a single, stable isotope of atomic mass equal to 196.967 g mol^{-1}, gold has an electronic configuration of [Xe] $4f^{14}$ $5d^{10}$ $6s^{1}$. Common oxidation states include 0, 1, 3 and the less common oxidation states of

2 and 5 have been documented for complexes containing only one gold atom. However, many complexes with gold-gold bonds, in which it is difficult to assign formal oxidation states to the gold atom, have also been noted.[37, 38]

The gold metal is the most noble of metals which can react with various oxidising agents at ambient temperatures, provided a good ligand is present to lower the redox potential below that of water. Attack by most acids, under ordinary conditions, does not occur for the gold metal and it is also stable in basic media. However, gold does dissolve in 3:1 hydrochloric-nitric acid (*aqua regia*) to form $HAuCl_4$ and in alkaline cyanide solutions in the presence of air or hydrogen to form $[AuCN]^-$; these reactions are important to the extraction and refining of the metal.

Redox potentials, in the absence of co-ordinating ligands, are useful for understanding the nobility of gold; thus for gold to react it must be oxidised e.g. $Au \rightarrow Au^+ + e^-$. The tendency for this reaction to take place is given by the Nernst equation (see Equation (1.3)), where E° is the standard electrode potential, estimated to be + 1.70 V, versus the normal hydrogen reference electrode, for gold at 25 °C, and the equation is reduced to:

$$\mathbf{E = 1.70 + 0.059 \log_{10} [Au^+]} \quad \text{..... Equation (1.4)}$$

Similar expressions may be derived for alternative oxidation processes:

$$\mathbf{Au \rightarrow Au^{3+} + 3e^-}$$

$$\mathbf{\text{where } E = 1.50 + 0.020 \log_{10} [Au^{3+}]} \quad \text{..... Equation (1.5)}$$

$$\mathbf{Au + 3H_2O \leftrightarrow Au(OH)_3 + 3H^+}$$

$$\mathbf{\text{where } E = 1.46 - 0.059.pH} \quad \text{..... Equation (1.6)}$$

$$Au(OH)_3 \leftrightarrow AuO_2 + H^+ + e^-$$

$$\text{where } E = 2.63 - 0.059.pH \quad \ldots\ldots \text{ Equation (1.7)}$$

The above reaction systems can be expressed graphically in the Pourbiax diagram, in which the equilibrium potential of each couple is plotted against the pH for a particular concentration; as illustrated in Figure 1.3.

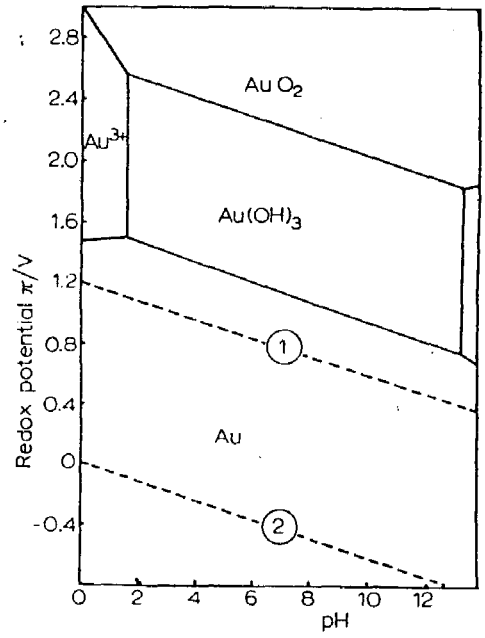

Figure 1.3 A simplified Pourbiax diagram for gold.

(Adapted from: 'The chemistry of gold', by R. J. Puddephatt.[38])

A simplified Pourbiax diagram for gold (concentration of the order of 10^{-4} mol dm^{-3}), as shown in Figure 1.3, indicates the conditions under which particular species are expected to be formed. The dotted lines (1) and (2) represent the stability limits for water.

The oxidised forms of gold only exist at potentials greater than for line (1). Under these conditions, water will be oxidised to oxygen and the various oxidised gold species will be reduced to gold metal. In the absence of co-ordinating ligands, gold cannot be oxidised by

dissolving oxygen in the presence of either strong acids or alkalis. Gold is thus, truly the noblest of the metallic elements.[38]

1.3.3 The advantages of electrochemistry as a detection tool for gold ions:

Gold is one of the first three metals known to man, however the quantitative and qualitative determination of gold ions with accuracy, sensitivity and selectivity remains an ongoing topic of interest.[35, 36] The method chosen to determine the gold depends on the state of the sample to be analysed, the expected gold content and the interfering impurity levels. Many of the available methods require the conversion of the gold sample to a soluble form such as $[AuCl_4]^-$. Gravimetric and titrimetric assays provide useful bulk assay methods in determining the presence of gold ions, such as from the ore sample.

Spectrophotometric methods have a lower detection limit and may even be useful in trace element analysis. However, to avoid interferences by other metal species, the technique requires the extraction of the gold ions into an organic solvent, or in other methods various reagents, such as Rhodanine, need to be added to the sample to form coloured complexes, which can then be viewed in the visible spectrum. Another disadvantage of the spectrophotometric method, is that the oxidation state of the gold ion is not distinguishable from this method.[37 - 39]

Emmission spectrography, atomic absorption and neutron activation instrumental techniques are all particularly useful for the determination of trace quantities of gold. The trace level detection limits, however, require the use of these techniques in conjunction with solvent extraction methods. Emission spectrography can detect small quantities of gold in a metal sample in the range of 0.5-20ppm, as well as the purity level of the gold in the sample.

Atomic absorption techniques have a detection limit in the region of 10^{-8} mol dm^{-3} in solution and tend to obviate extensive sample preparation. Disadvantages of the atomic absorption technique include the susceptibility of this technique to the presence of other interfering ions, the sensitivity to pH and rigid experimental parameters (which may be overcome with the use of the solvent extraction technique) and the inability to determine the oxidation state of the gold ion in solution.

Neutron activation has been hailed as one of the most sensitive techniques for gold analysis with accurate determination levels of 1 ppb. The sensitivity arises from the high-neutron capture cross sections of the only natural isotope, ^{197}Au. The disadvantage of this technique includes the sample preparation and the result of β and γ emmisions. The neutron activation technique is thus, not suited for studying the interaction of metal ions and biological substrates.[37, 38, 40]

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) offers another alternative to the determination of gold ions in solution. The technique has been used in a wide variety of applications, including biological fields and is useful in the speciation detection of the gold samples. The ICP-MS technique is a combinatorial technique and thus, posses enhanced sensitivity, accuracy and selectivity in comparison to any of the single techniques mentioned above.

However, the compromise for the advantages of the ICP-MS technique is in the form of the sharp increased cost and the lack of portability of the equipment. Detection limits tend to vary depending on the sample and the context (typically around 10^{-9} mol dm^{-3}), however, a limitation of the ICP-MS technique is that the sample must contain less than 0.2 % total dissolved solids, since the nebulizer becomes blocked easily. Dilution of the sample may overcome this problem, but a loss in sensitivity is then experienced.[41, 42]

Electrochemistry on the other hand, a single technique, offers the advantages of high sensitivity, selectivity toward electroactive species, portable and fairly low cost instrumentation, speciation capability, rapid multi-component determinations without requiring metal enrichment techniques and a wide range of electrodes that allow assays of unusual environments.[30, 43] Extremely low (nanomolar) detection limits can be achieved with very small (5 - 20 ml) sample volumes, thus allowing the determination of analyte amounts of 10^{-13} - 10^{-15} mol dm^{-3} on a routine basis (depending on the type and context of the analyte).

Electrochemical metal ion speciation is unique for that ion and its oxidation number, since there is only one assigned redox peak potential for a redox couple. The simultaneous detection of other metal ions in solution is possible, provided the metal ions do not posses overlapping peak potentials. Electrochemistry depends on the electrolytic deposition step for the preconcentration of trace components and thus has the advantage of minimising the risk of contamination, since little or no reagent has been added. A further advantage of this technique is the flexibility of combinations with other detection techniques, such as the chromatographic or optical procedures, resulting in even further enhanced sensitivities.[30, 31, 33, 34, 37]

1.3.4 Problems associated with the electrochemical detection of gold:

A major problem in the electrochemical stripping analysis for gold(III), and other oxidation states of the gold ions in solution, is to find a suitable electrode onto which elemental gold can be deposited for subsequent stripping. The deposition of elemental gold onto the carbon or platinum electrode is hampered by the slow nucleation process.[44] The nucleation and growth process, during the metal deposition, has been shown to be pH, complexing agent and potential dependent.

The temperature and nature of the substrate are also important factors and changes in either provoke modification of the type of nucleation obtained. For example, at temperatures below 50 °C there is a progressive nucleation and growth process observed, however, above this temperature, crystal growth occurs without new nucleation formation and is indicative of instantaneous nucleation processes.[45]

Attempts have been made to deposit gold (from a standard gold solution in which the concentration and oxidation state of the metal ion was known) directly onto a platinum or glassy carbon electrode, using pulsed potential electrolysis. The precision obtained was satisfactory, as would be expected from a relatively pure substance, but the accuracies were poor. The reason for this, as seen with the silver ions, may be attributed to the slow initial nucleation process on the working electrode surface. In order to obtain reproducible deposition results for gold(III), a concentration exceeding 200 $\mu g\ l^{-1}$ of gold ions in solution should be maintained.[44]

Another problem associated with the electrochemical determination of gold ions, is the natural presence of interfering cations such as copper(II), silver(I), mercury(I) and iron(III) in the gold sample. The interfering cations, as well as some interfering anions, may be present in the sample solution and will compete for electrode surface space during the preconcentration step and/or complex with the gold ions in solution. Solvent extraction chemistry has largely solved the problem of interfering ions. However, the solution is both costly and time consuming to industry, not to mention the complications that arise in the analysis of complex samples such as mining effluents, making this a rather unattractive option.[45, 46]

Apart from the chemical problems associated with the nature of gold atoms, practical experimental problems occur as well. The choice of the electrode will affect the detection limits of gold ions in solution. Common electrode choice includes the carbon fibre, platinum, gold, glassy carbon, carbon paste and modified carbon paste electrodes.

The unmodified electrodes are plagued by the nucleation problem, whilst modified electrodes are not easily regenerated and are often time consuming with the preparation time before numerous experiments can be run one after each other. Carbon paste electrodes tend to lack sensitivity and selectivity, however, the modified electrodes usually make up for this deficit, but at the cost of effective regeneration of a clean electrode surface and hence reliability of the peak obtained.[44 - 46]

Noble metal electrodes are inclined to be lacking in reproducibility of the stripping peaks, both quantitatively and qualitatively. The oxide film formation, in oxidising and non-complexing media, (as in the case of gold) has been the reason attributed to the irreproducibility of the stripping peaks.[47] An example of this is the polycrystalline gold disc electrode in aqueous acidic media, which undergoes oxidation at its surface to Au_2O_3 in the presence of water and sulphuric acid.[48] The platinum noble metal electrode is more likely to fall prey to the chloride film formation at its surface in the presence of hydrochloric acid.

In constant current stripping analysis experiments for gold(III) ions on carbon fibre and platinum electrodes, both carbon and platinum electrodes have been shown to work equally well in the determination studies. However, the carbon fibre is preferred of the two electrodes studied; since the platinum electrode has the ability of forming inter-metallic compounds at its surface (e.g. antimony) and the hydrogen over-potential is also higher on the platinum electrode.[45, 46]

Consequently in addition to the usual parameter choices in electrochemical experiments, (electrode choice, stirring rate, deposition times and selected potentials *etc.*) one has to take into account the effect of the gold nucleation problem and the problems of the other metal ions associated with the gold. The stripping solution (or electrolyte) present, the chosen electrochemical detection technique and the presence or absence of co-deposition ions will all effect the determination of gold(III) ions.

1.3.5 Electrochemical stripping techniques applied:

The quantitation of trace and ultratrace components in complex samples of environmental, clinical or industrial origin represent an important task of modern analytical chemistry. In the analysis of such dilute samples, the employment of some type of preconcentration step, prior to actual quantitation, is required. The main objective of the preconcentration step is enrichment of the sample, however, the step may also serve to isolate the analyte from a complex matrix and hence result in improved selectivity and stability.

Electrolytic deposition represents an efficient method for the enrichment and isolation of trace components. Stripping analysis is the best-known analytical technique that incorporates an electrolytic preconcentration step. The technique couples the advantages of extremely low detection limits ($\sim 10^{-10}$ - 10^{-11} mol dm^{-3}), multi-element and speciation capabilities, suitability for on-line and *in situ* measurements and a low cost factor.[30, 31]

Anodic Stripping (Linear sweep) Voltammetry (ASV):

Anodic stripping voltammetry characteristically involves the reduction of a metal ion to the metal form which is then dissolved in a small volume of mercury (characteristically a thin mercury film or a hanging mercury drop) to form a metal-mercury amalgam. The formation of a metal-mercury amalgam signifies the end of the preconcentration (or accumulation) step. The preconcentration of the metal is accomplished by cathodic deposition at a controlled potential and time. The deposition potential is at least 0.3 - 0.5 V more negative than E^0 for the least easily reduced metal ion to be determined.

The stripping step follows the preconcentration step. The positive-going potential scan results in the oxidation of the metal in the amalgam and the stripping of the amalgamated metal out of the electrode, in an order that is a function of the standard potential of that metal. The stripping step may be carried out in a linear fashion, referred to as linear sweep

stripping voltammetry, or in a more sensitive mode known as a pulsed, or a potential-time wave form, technique.[49] The diagrammatic representation of the linear sweep potential scan anodic stripping voltammetry process is illustrated in Figure 1.4.

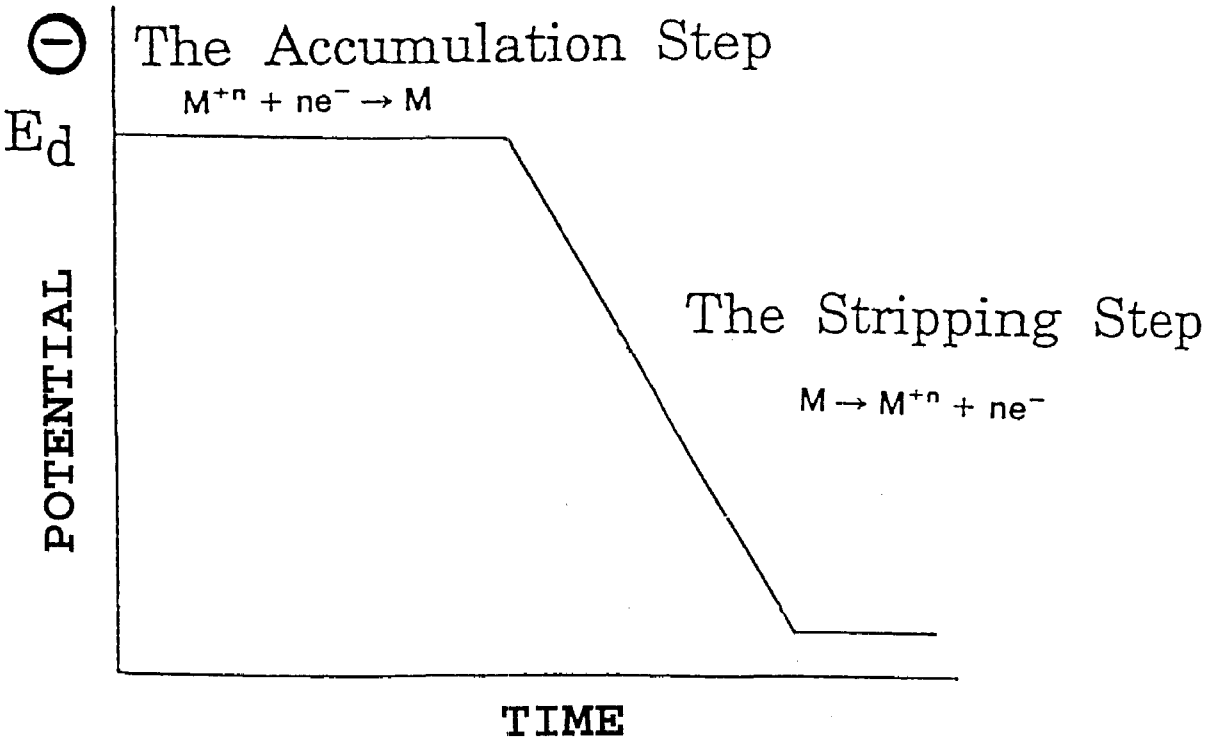

Figure 1.4: An illustration of the Anodic Stripping Voltammetry technique.

(Adapted from: 'Analytical Electrochemistry' by J.Wang.[30])

Mercury is not considered to be a suitable electrode material for the determination of gold ions by anodic stripping voltammetry, as mercury has an oxidation potential considerably less positive than that of gold and a tendency to dissolve in the gold substrate.[31] The gold accumulated at the electrode will always oxidise at more positive potentials than mercury, irrespective of the complexing agents present in the stripping solution. A suitable organic solvent, in which gold is oxidised cathodic to mercury, has not been found as yet.[44]

However, attempts made to deposit gold directly onto platinum and carbon type electrodes are often hampered in the preconcentration step as a result of the slow nucleation process occurring at the electrode surface. A compromise on the conventional ASV technique has been developed by Huiliang *et al.*.[44] Pulsed potential electrolysis was applied and the technique required the co-deposition of mercury and gold onto the working electrode, followed by the intermittent re-oxidising of the mercury, permitting the gold to be unoxidised and attached to the electrode surface.[44]

In the pulsed potential experiments, conducted by Huiliang *et al.*, two peaks were observed in the final stripping run.[44] The two peaks were clearly separated and the peak due to gold(III) could easily be determined with greater reliability than that observed with the unmodified carbon or platinum type electrodes.[44, 50] Gold, to date, has not been routinely determined by anodic stripping voltammetry; particularly in the commercial laboratories since the omission of mercury from ASV for gold detection has resulted in unsuitable precision and detection limits.[51]

However, with the success of the mercury and gold co-deposition experiments by Huiliang *et al.*[44], conventional linear sweep potential scan ASV (*i.e.* utilising mercury) was attempted in this study for the detection of a known concentration of gold(III). The study is aimed at applying the refined ASV technique to a mine sample for the benefit of the South African mining industry.

Adsorptive Cathodic Stripping Voltammetry (AdCSV):

Adsorptive stripping analysis greatly enhances the scope of stripping measurements toward numerous trace elements. The strategy is relatively new and involves the formation, adsorptive accumulation and reduction of a surface active complex of the metal. A negative-going potential scan or a constant cathodic current can be employed for measuring the adsorbed complex.

Reduction of the metal species in the adsorbed complex is most common, however, the possibility of reduction of the ligand may also be exploited.[30] Redox speciation may also be obtained from the adsorptive stripping voltammetry experiments, since any oxidation state can be accumulated unlike the metallic state in the case of anodic stripping voltammetry.[49] The adsorptive cathodic stripping voltammetry process is illustrated in Figure 1.5 and the stripping step is depicted in the square wave potential scan format.

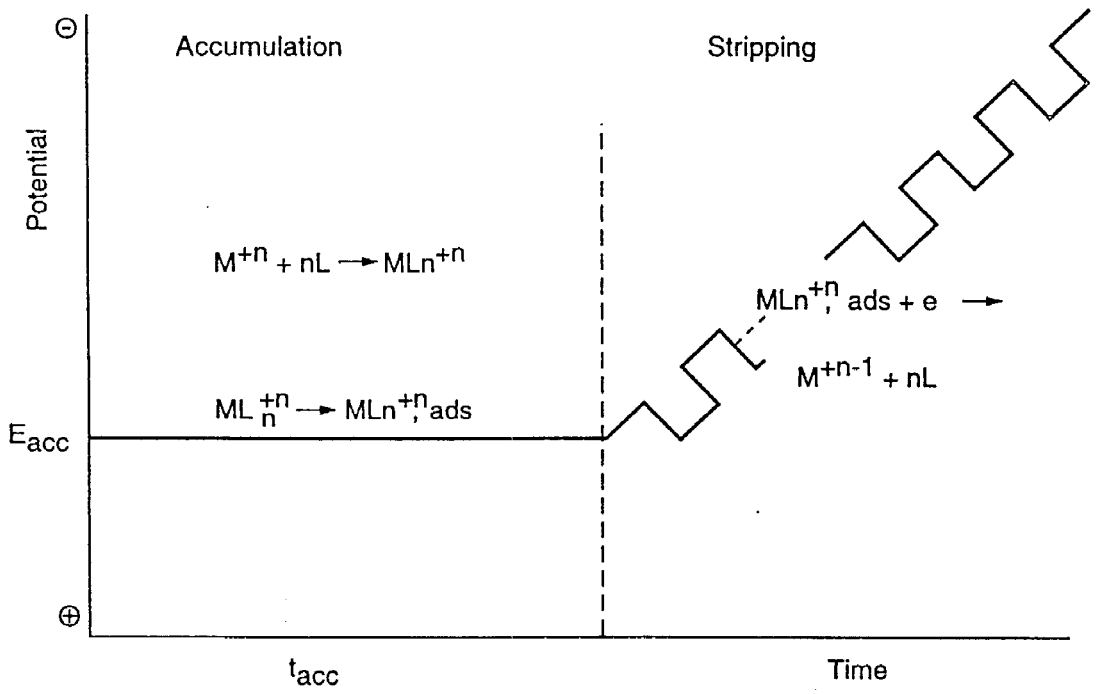

Figure 1.5: The accumulation and stripping steps in the adsorptive cathodic stripping measurements of a metal ion(M^{+n}) in the presence of a ligand (L).

(From: 'Analytical Electrochemistry', by J.Wang.[30])

The selectivity of the chemical step (complex formation) can be used to increase the overall selectivity of the analysis. A ligand capable of binding only a few metals should make possible the formulation of a highly selective scheme, whilst controlled adsorptive accumulation at stationery electrodes permits substantial enhancements of the electrode surface concentration of the complex.

Apart from extending the scope of stripping voltammetry, the metal complex adsorption approach offers alternative, and often improved, schemes for measuring 'difficult' metals. The term "difficult' metals refers to the group of metals that have extreme redox potentials, may form inter-metallic compounds or that suffer from poor selectivity.[31, 52]

A need to develop ligands that may increase the sensitivity and selectivity of metal ion determination has been expressed.[52] Ligands such as catechol and oxine have been documented for the detection of a wide range of metal ions including copper, iron, molybdenum, uranium and tin.[30, 52] A substituted form of resorcinol, 4-(2-Pyridylazo) resorcinol, was employed in the successful spectrophotometric determination of gold(III).[53]

However, to date, there have been no adsorptive cathodic stripping studies completed on any of the yeast cell wall extracts as possible ligands for gold(III) determination. Linear sweep- and the square wave- potential scan adsorptive cathodic stripping voltammetry were utilised in this work, to investigate the relationship between mannan (a polysaccharide yeast extract) and gold(III) in the bioremediation studies completed. The aim of this work is to better understand the gold ion uptake by the yeast *S. cerevisiae*.

Square Wave Stripping Voltammetry:

Square wave voltammetry was invented in 1952 by Barker, but little use was made of this technique at the time owing to difficulties with the controlling electronics. Advances in instrumentation has allowed this technique to become an important tool in analysis.[31] Square wave voltammetry is defined as a large-amplitude differential technique in which a wave form composed of a symmetrical square wave, superimposed on a base staircase potential, is applied to the electrode.[30, 54]

A variety of square wave forms exist and this has lead to a fair amount of confusion, since the wave forms employed have simply been described as square waves. There are in fact three main types of square wave formats namely: Barker, Kalousek and Osteryoung employed in square wave voltammetry.

The Barker format is the simplest to visualise since the wave form is a direct analog to the sinusoidal ac voltammetry form. The format employs a symmetric square wave of frequency 'f' and amplitude 'dE' riding on either a ramp or slow staircase wave form. Barker square wave methods are characteristically employed in conjunction with mercury drop electrodes and multiple cycles of this type of square wave is applied to each drop of mercury.

The Kalousek method is characteristically employed in square wave polarography and is termed the Kalousek Type III. Kalousek formats employ a lower frequency method which measures the current only on the reverse half wave cycle of the square wave. Extreme sensitivity to the electrochemical reversibility of the couple and the chemical stability of the product of the forward step are known characteristics of the Kalousek Type III form.[55]

The most common form of square wave today was first proposed by Ramaley and Krause, however, this form is most closely associated with the Osteryoungs as a result of their many publications in this field.[56, 57] The Osteryoung wave form, as it is now termed, differs from the other two formats in that the base potential (potential of the staircase) increases by 'dE' for each full cycle of the square wave whose half height is 'E_{sw}' and whose period is 'τ'. The Osteryoung wave format may be applied to stationery, as well as drop electrodes.[58]

The general response obtained from square wave formats is the difference between the current sampled at the end of the forward pulse and the current sampled at the end of the reverse pulse within a given wave form cycle. The diagrammatic representation of the square wave voltammetry technique is illustrated in Figure 1.6.

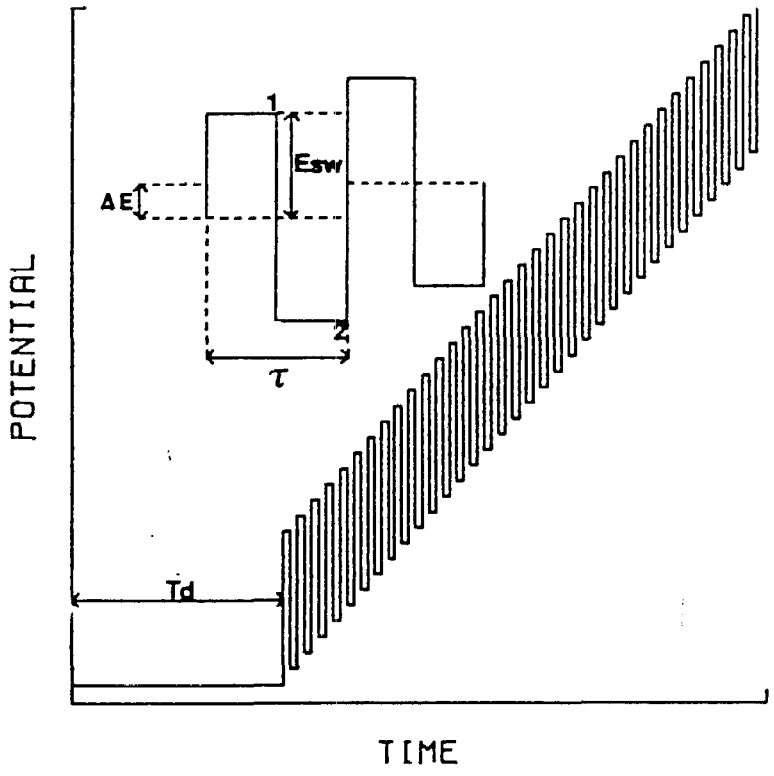

Figure 1.6: Excitation signal for square wave potential scan voltammetry.

(Adapted from: 'Analytical Electrochemistry', by J.Wang.[30])

Advantages of the square wave technique include enhanced sensitivity over linear sweep potential scan forms, greater speed in analysis and reduced problems with the blocking of the electrode surface compared to the differential pulsed techniques.[31] Barker and Osteryoung square wave potential scan stripping voltammetry was applied to the determination of the gold-mannan complex, in an effort to enhance the detection limit of gold ions in solution.

1.3.6 The modification of electrodes:

At a modified electrode, the electrode surface has been deliberately altered by adsorption, physical coverage or by the bonding of specific species to the surface.[31] Chemically modified electrodes comprise a relatively modern approach to electrodes systems, which have found numerous uses in a wide spectrum of basic electrochemical investigations and in the design of electrochemical devices for chemical sensing. The key to electrode modification is to build in chemical sensitivity and selectivity into the electrode function. The detection of the analyte of interest, will be selective by the modified electrode and aim for the exclusion of other unnecessary constituents and reactions.[30, 31, 59]

Electrodes which have an electroactive mediator attached or incorporated into them are termed chemically modified electrodes. Modified electrodes often give rise to currents that are higher than in the absence of a modifier and in certain instances the modifier, in a supporting electrolyte alone, displays voltammetric characteristics of the immobilised species. Several methods exist for the preparation of a chemically modified electrode; these include the drop-dry method, electrochemical deposition and the manufacture of carbon paste and modified carbon paste electrodes.[59, 60]

The Carbon Paste and Modified Carbon Paste Electrodes:

The carbon paste electrode (CPE) was invented to be used in positive potential ranges where mercury electrodes are not applicable, as well as to act as a renewable carbon electrode surface. Carbon paste electrodes tend to demonstrate lower background currents compared to solid graphite or noble metal electrodes.[61, 62] Typically detection limits for carbon paste type electrodes range in the order of 1.00×10^{-9} mol dm^{-3}. However, a disadvantage of carbon paste electrodes is the reproducibility factor compared to mercury electrodes or sensors made of compact materials (noble metals or glassy carbon), but the reproducibility is quoted to be better than other types of solid carbon electrodes.[31, 63]

Carbon paste consists of a mixture of carbon/graphite powder with a liquid binder and is prepared by mixing the two components together. The usual particle size of graphite materials ranges between 5 and 20 μm; larger particles produce a more rough texture and unfavourable mechanical and electrochemical properties. The required properties of the particulate component of the paste are uniform particle size distribution, high chemical purity and low adsorption capability for oxygen and electroactive impurities. The binder or pasting liquid should be electroinactive and chemically inert, immiscible with the analyte solution, minimally volatile and free of electroactive impurities.[63]

Unmodified carbon paste electrodes are frequently employed in routine electroanalytical chemistry, mainly for stripping analysis after deposition of analytes as metals, (e.g. gold or silver) or as insoluble oxides (e.g. manganese or lead). However, on the addition of a modifier to the paste electrode improved detection limits, along with enhanced selectivity of the interested species, have been observed. In most instances, due to the simplicity of the procedure, the modifier is added directly to the paste material.[63]

Modified carbon paste electrodes (MCPE's) have found use in a variety of applications. Wang *et al.* were the first to utilise biomass modified electrodes for the accumulation of analytes in 1988.[63, 64] The algal biomass extracts were employed for the preconcentration of gold and copper ions from solution.[22, 63 - 65] In this study cathodic stripping voltammetry and MCPE's were combined with the aim of exploring the metal-ligand interactions of gold(III) and yeast cell wall extracts.

1.4 Computer Modelling, Nuclear Magnetic Resonance and Infrared Spectroscopy as techniques for studying metal-biomass interactions:

1.4.1 Computer Modelling:

Advances in computing, in particular the ready availability of high-resolution graphics, have greatly increased the interest in computer-based molecular modelling. Molecular modelling is now widely used as an aid in the interpretation of experimental results and in the design of new materials including the study of the interaction of metal ions with biological substrates. The fundamental assumption underlying the molecular mechanics method, is that the positions of the atoms of a molecule, ion, solvate or crystal lattice are determined by forces between pairs of atoms, (bonds, van der Waals interactions, hydrogen bonding and electrostatic interactions) and between groups of three (valence angles) and groups of four (torsional angles, planes) atoms.[66]

Two types of information are obtained from any molecular mechanics experiment: the minimum value of the strain energy and the structure associated with that minimum. However, the modelling of large biomolecules, and their interactions with metals, is fraught with difficulties. The major problem arises from the flexibility of the molecules, resulting in a manifold of adopted conformational geometries.

However, despite the difficulties with biological models, when no unequivocal determination of a structure is available by experimental methods, then structure prediction may be the only means of obtaining a three-dimensional model of the molecule. In metal-macromolecule adducts this is often the case and structures obtained by molecular modelling can be a genuine aid in the visualisation of these interactions.[66]

Full scale molecular modelling of the mannan (yeast cell wall extract) was not attempted in this study (and no form of visual modelling of yeast extracts have been completed to date).

However, the study aims at a graphical representation of the molecule with a minimised energy configuration. A visual picture of the energy minimised gold-mannan complex and the type of gold co-ordination to the mannan was obtained. The study will thus contain the first known visualisation of a gold-yeast interaction.

1.4.2 Nuclear Magnetic Resonance Spectroscopy:

Nuclear magnetic resonance (NMR) spectroscopy over the past few decades has become a very powerful tool for the organic chemist and additionally revolutionised the study of natural products. The careful choice of one- and two- dimensional experiments has efficiently elucidated the structure of several complex biological molecules. However, the technique is only applicable to those nuclei which possess a spin quantum number, *I*, greater than zero. The most important of such nuclei are the ^{1}H and ^{13}C nuclei, although, ^{31}P and ^{14}N have become increasingly important as more biological-type experiments are being performed.[67, 68]

The basis of the NMR experiment is to subject the nuclei to radiation, which will result in a transition from the lower energy state to a higher level. The precise difference in energy levels between the two spin orientations is dependent on the particular location of the atom of the molecule, since each nucleus is subject to the differing effects of the magnetic fields of neighbouring nuclei. Only nuclei which are in exactly the same magnetic environment will have exactly the same energy difference between spin orientations, when placed in a magnetic field. In NMR spectroscopy these differences in energy are detected and provide information on the variety of locations of the nuclei in the molecule.[67]

By convention, frequency, and therefore magnetic field strength, increase from left to right in the NMR spectrum. Tracing the spectrum from left to right is referred to as moving upfield, whilst moving from right to left is a downfield shift. Upfield absorptions are said to be more shielded and the downfield absorptions are the result of deshielding. The position

of an absorption peak in the NMR spectrum may be represented either on a frequency scale (Hz), or on the scale of magnetic field (tesla) and by convention the frequency scale is used. However, in order to make direct and rapid comparisons between spectra recorded on instruments operating at different frequencies, the positions of absorptions are normally quoted on the σ scale, which is independent of the instrument operating frequency. The σ value is obtained by dividing the position in Hz by the instrument frequency (in MHz) and is expressed in parts per million (ppm).[68]

The study of molecular structure, conformational changes, interactions of biological molecules with various substrates and certain types of kinetic investigations, are the primary uses of NMR in the biological field. The studies are carried out in solution and at present there appears to be an upper molecular mass limitation of 20 000 Daltons in the study of biological macromolecules. The major reason for the limitation is the large number of protons in similar structural environments, resulting in numerous absorption peaks in a similar region.[69]

NMR spectroscopy is a non-destructive technique and structural determinations may be carried out on less than 1 mg of sample. Developments in the field of NMR spectroscopy over the last two decades have completely altered the approach towards structural elucidation, in particular of the complex polysaccharides. The applications of this technique are numerous and advantageous, especially when studying interactions of polysaccharides and metals.

The basic configuration of the sugar residue may be determined from the characteristic proton coupling pattern displayed by the molecule. The coupling constant is customarily large (5 - 8 Hz) when the protons are transdiaxial and small (1 - 3.5 Hz) when the protons are gauche. Values for the first four vicinal coupling constants (H-1 to H-4) are sufficient to establish the basic configuration of the sugar.

The one-dimensional (1D) experiment involves the excitation of a single type of nucleus, ^{1}H and ^{13}C being the most common. A typical 1D ^{1}H NMR spectrum of a microbial polysaccharide for example is fairly complex, due to the degree of overlap of signals arising from ring protons. However, despite the complexity of the ^{1}H NMR spectrum, a great deal of preliminary information may still be obtained.

Characteristically the spectrum of a microbial polysaccharide may be divided into three broad regions, *viz.* the anomeric region (δ 4.5 - 5.5), the ring proton region (δ 3.2 - 4.5) and methyl group region (δ 1.2 - 2.3).[67, 70] The anomeric protons of the constituent monosaccharides are significantly deshielded; due to their proximity to the ring oxygen and consequently resonate downfield from the rest of the protons in the compound. The anomeric region yields information concerning the size of the repeating unit, the possible identity of the constituent monosaccharides and their anomeric configurations. The area underneath each anomeric signal is proportional to the number of protons represented, revealing the size of the repeating unit.

Two-dimensional (2D) experiments are more efficient for the simultaneous determination of a large number of spin correlations. All 2D experiments involve the use of a multiple pulse sequence containing a variable delay t, between pulses in which free induction decays $S(t_2)$ are measured for an evenly spaced series of values of t_1 to build up a matrix of data $S(t_1.t_2)$. The most commonly used 2D experiment is ^{1}H-^{1}H homonuclear shift correlated spectroscopy (COSY), which is frequently used for polysaccharide determination.

In the COSY experiment the basic pulse sequence involves the application of a 90° pulse to the sample, followed by a delay period (t_1), during which the spin system evolves as it would in a normal free induction decay. A second 90° pulse, the mixing pulse, interrupts the evolution, followed by a second time period t_2, which allows the evolution of the spin system resulting in a detected and recorded free induction decay signal.

Double Fourier transformation results in two 1D spectra, which can be plotted at right angles to each other resulting in a COSY contour plot. The diagonal of this plot represents the 1D spectrum.[68, 69]

The aim of the NMR experiments in this study is to obtain a defined structure of the mannan extract and to substantiate any electrochemical data obtained for the gold-mannan interactions.

1.4.3 Infrared spectroscopy:

Infrared (IR) spectroscopy can be described as the use of instrumentation in measuring a physical property of matter, and the relating of the data to chemical composition. The instruments used are called infrared spectrophotometers, and the physical property measured is the ability of matter to absorb, transmit, or reflect infrared radiation.

Infrared spectroscopy is a non-destructive type of analysis (the sample can normally be recovered for other use), and is useful for microsamples.[71] The region of infrared spectrum which is of greatest importance to the organic chemist is that which lies between 4000 and 660 cm^{-1}. Metal samples may be studied in the lower region of the spectrum. Absorption bands in the spectrum result from energy changes arising as a consequence of molecular vibrations of the bond *stretching* and *bending* (*deformation*) type. The positions of atoms in the molecule may be viewed as the mean equilibrium positions of atoms in molecules, whilst the bonds between atoms are analogous of springs, subject to stretching and bending.

Hydrogen or carbon bonded to oxygen or nitrogen, for example, give rise to strong infrared absorption patterns because of the polarity of these particular bonds. In contrast, no absorption results from stretching vibrations in a homonuclear double or triple bond which is symmetrically substituted; such vibrations are termed *infrared inactive*. The recognition of such bonds is possible by an examination of the Raman spectra of these molecules.[68]

Adjustments of the spectrometer to obtain optimum peak efficiency, has allowed for the studying and characterisation of the chemical components of many micro-organisms. Spectra of extracted polysaccharides have even helped in the differentiation of species types and sub-types of a particular micro-organism. The infrared spectra of many sugar complexes have been recorded both in the solid and aqueous states. The addition of metals to the sugar system characteristically results in the lack of the bands in the expected sugar region, due to the co-ordination of the polysaccharide to the metal.[70, 72] Infrared spectroscopy is thus a useful technique to further investigate metal-biomass interactions, as aimed at by this study.

1.5 Overall Research Aims:

The aim of the present research project is three-fold. (i) The design of a system whereby gold ions may be detected; utilising an electrochemical technique known as stripping voltammetry on an unmodified glassy carbon electrode. (ii) The application of the designed system to a mining effluent for the detection of gold ion presence. (iii) Finally, to obtain a better understanding of the chemical interactions between biological matter (biomass) and the gold ions. Adsorptive cathodic stripping voltammetry on glassy carbon and modified carbon paste electrodes is proposed for the metal-ligand studies. NMR, IR and computer modelling techniques will be utilised to substantiate the data obtained from the electrochemical experiments.

Gold has been chosen as the primary metal of interest, since South Africa is a major metal ore mining and refining country and one of the chief metals mined is gold. The determination of gold ions with accuracy, sensitivity and selectivity remains an ongoing topic of interest. The successfully designed electroanalytical system, for the determination of gold ions in solution, would aim to determine the analyte with reasonable accuracy and precision levels (± 5 % deviation); sufficient sensitivity levels and preferential selectivity for the ions of interest, so as to apply the system to an industrial application.

The metal-biomass interaction aspect revolves around the determination of the specific components and the chemical functional groups of the cell wall matrix responsible for the metal binding. *S. cerevisiae* has the capacity to accumulate metals and since this yeast is economically viable for bioremediation applications, the cell wall components of this species were chosen for investigation.

The study further aims at investigating the effects of copper and silver ions on the metal-biomass interactions, since *S. cerevisiae* has been shown to bind these ions in significant quantities. Silver and copper ions are also commonly detected with the gold ore sample and the presence of the silver(I) and copper(II) may be detrimental for the binding of the gold-biomass complex.

The benefits of these findings would be useful both from a biological perspective, as well as for industry. Once, more is known about the particular binding sites on a micro-organism, microbiologists/biochemists have an outline to work towards in terms of the optimisation of these sites for precious metal recovery. Information on the behaviour of gold ions in solution with biomass will be achieved, which may have implications in other gold research fields.

EXPERIMENTAL METHODS

2.1 Reagents:

Gold(III) (in the form of tetrachloroauric(III) acid) was purchased from Sigma-Aldrich. The mineral acids utilised included nitric acid from Merck, hydrochloric acid from BDH Suppl. Ltd. and perchloric acid was obtained from Associated Chemical Enterprises. The mercury(II) was obtained from Merck in the form of mercury(II) nitrate.

The mine effluent was a by-product of the Deelskraal number 7 mine, which is part of the group of mines owned by the Goldfields Mining Company. The 'effluent' is sourced from the mine service water, which refers to water that has been used for the crushing process of the ore and recycled several times, after which the water undergoes a reclamation process and is then run off the plant as effluent.

Silver(I), in the form of silver nitrate and utilised in the metal ion interference studies, was obtained from Holpro, whilst the copper(II) (copper nitrate) was from the Saarchem (Pty) Ltd Company. In the production of carbon paste electrodes, the mineral oil was obtained from N.T. Laboratories and the graphite powder was a product of the Saarchem (Pty) Ltd Company. The commercially obtainable cell wall extracts of *S. cerevisiae* (*i.e.* mannan, glucan, chitin and chitosan) were purchased from Sigma Aldrich.

The deuterated solvent for the NMR experiments was D_2O, purchased from Merck Chemical Company. In solution IR studies, triply distilled deionised water was used as the solvent. Solid-state IR experiments made use of analytical grade potassium bromide from the Saarchem (Pty) Ltd Company. NMR and IR techniques both made use of nitric acid (Merck) to acidify the solutions.

2.2 Apparatus employed:

Electrochemical data was obtained on a Bioanalytical System BAS-100 B/W workstation, connected to a C-2 voltammetry cell stand. A borosilicate glass vial, (capable of holding 15 - 20 ml of liquid) with fitting top, was utilised as the sample container.

The three electrode system was employed in all electrochemical experiments; as illustrated in Figure 2.1. The silver/silver chloride (KCl = 3 mol dm^{-3}) served as the reference electrode, whilst platinum wire was chosen as the auxillary electrode. Glassy carbon, platinum, carbon paste and modified carbon paste electrodes were all employed as working electrodes depending on the particular experiment.

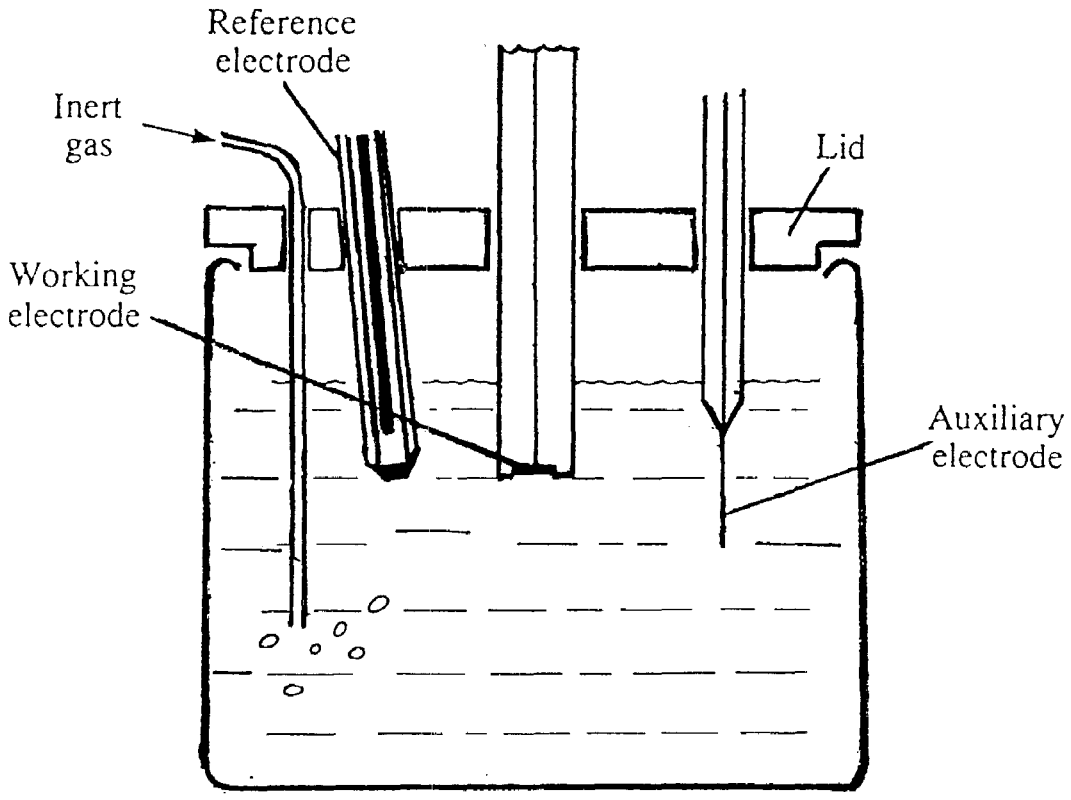

Figure 2.1: An illustration of the electrochemical cell utilising the three-electrode scheme.

The glassy carbon and platinum electrodes, commercially obtained from Bioanalytical Systems, were of 3.00 mm and 1.60 mm respectively in diameter. Borosilicate glass tubes (due to the relatively low metal-cation-binding property of this glass type), with an internal diameter of 1.10 mm, were cut to appropriate lengths to be used for the manufacture of the carbon paste electrodes.

A Mettler Toledo MP220/225 digital pH meter was employed for all pH investigations. The instrument was calibrated using a pH 7.00 and pH 4.00 buffer respectively. The temperature control function was set at the current room temperature, measured with a thermometer, at the time of the experiment.

A Jeol JSM 840 scanning electron microscope was employed for the scanning electron micrographs obtained, whilst the NMR experiments were recorded at 400 MHz on the Bruker AMX pulse Fourier transformed spectrometer. Samples were prepared using deuterated solvents and 5 mm tubes throughout. Unless otherwise stated, the probe temperature for all NMR experiments was 30 °C ± 1 °C.

Infrared spectroscopy experiments employed the Perkin Elmer Spectrum180 IR spectrophotometer for the studies of certain samples made up in KBr discs, using the pressed disc technique. The Fourier transformed infrared (FTIR) apparatus, used for the solution-phase and KBr disc experiments, was a computerised Perkin Elmer Spectrum 2000 IR spectrophotometer. The fully assembled, sealed IR cell, with a calcium fluoride window, was chosen for the solution phase experiments. The sealed IR cell has a fixed path length, which uses an amalgamated lead spacer to form a permanent leak proof seal.

The computer modelling technique was employed to obtain a visual picture of the complexes. MSI Cerius2 version 3.5 software, was run on a silicon graphics computer for all the modelling experiments. The registered Hyperchem® software package provided the vehicle for further viewing of the molecule in either a space-filling- or stick model form.

2.3 Experimental procedure:

Clean glassware is essential for the analytical analysis techniques. In all experiments performed, the glassware had been washed in detergent and rinsed several times before being soaked in nitric acid for at least 24 hours; followed by further washing in distilled deionised water. Reagents employed were all either of analytical grade (AnalaR) or reagent grade and were utilised without further purification.

Triply distilled deionised water was used for all experimental processes and diluted solutions were prepared daily from the stock solution. The gold(III) stock solution, of concentration 1.00×10^{-3} mol dm^{-3}, was prepared in an acid mixture of 1 : 1 ratio of HCl and HNO_3 (both 0.10 mol dm^{-3}). The diluted gold(III) solutions were prepared by adding the appropriate volume of gold(III) from the stock solution (1.00×10^{-3} mol dm^{-3}) to a volumetric flask and filling the flask to the mark with nitric acid (0.10 mol dm^{-3}). The copper(II) and silver(I) solutions were prepared with HNO_3 (0.10 mol dm^{-3}), whilst the mercury(II) (1.50×10^{-3} mol dm^{-3}) and mannan (1 mg ml^{-1}) stock solutions were prepared from triply distilled deionised water.

A minimum purge time of 5 minutes, with nitrogen gas and simultaneous solution stirring, was employed for the electrochemical experiments. The reason for purging the solution is to minimise any oxygen effects that may occur; particularly at very positive potentials which are often observed for gold ion determination. The potentials quoted are cited with reference to the Ag/AgCl reference electrode for all data accumulated.

Prior to each run, the glassy carbon electrode was dipped in dilute nitric acid and the electrode surface was then cleaned by polishing with alumina on a Buehler felt pad, followed by rinsing in triply distilled deionised water and finally being rinsed in the chosen electrolyte of the experiment. The platinum electrode underwent similar treatment. However, soaking in a hydrogen peroxide solution, before polishing, for 10 minutes was

also employed in the regeneration of the electrode surface; particularly in cases where the reproducibility of the stripping peak was problematic.

2.3.1 Anodic stripping voltammetric determination of gold(III):

Anodic stripping voltammetry (ASV) experiments were performed on the gold samples with a glassy carbon electrode chosen as the working electrode for the experiments. The presence of excess mercury ions (concentration ranging between 1.50×10^{-4} to 9.00×10^{-4} mol dm^{-3}) was ensured by adding between 2.5 ml and 9 ml of a mercury(II) nitrate stock solution (1.50×10^{-3} mol dm^{-3}) to the sample vial. The gold(III) concentration ranged from 6.67×10^{-7} to 4.00×10^{-6} mol dm^{-3}, by the dilution of 1 to 6 ml of gold(III) stock solution (1.00×10^{-5} mol dm^{-3}) with nitric acid (0.10 mol dm^{-3}) to a 15 ml total volume.

The deposition potential of 0.10 V was employed for 100 seconds for the anodic stripping voltammetric determination of gold(III). The stir-rate for the magnetic stirrer bar was set at 3000 rev min^{-1} on the BAS-100 B/W workstation. The potential was scanned from 0.10 V to 1.50 V in a positive-going scan and at a scan rate of 100 mV s^{-1}.

The anodic stripping voltammetry technique, on an glassy carbon electrode (GCE) and in the presence of mercury(II), was applied to the determination of metal ions other than gold, present in the mine water sample. The deposition potential of -1.00 V for 300 seconds was applied in the accumulation step of the ASV experiment, whilst a scan rate of 100 mV s^{-1} was employed in the stripping step. A 5 ml volume of mine water sample was added to 2 ml of mercury ions (1.50×10^{-3} mol dm^{-3}) and 8 ml of nitric acid (0.10 mol dm^{-3}), to obtain a total volume of 15 ml. The prepared mine water sample was stripped in the negative potential window from -1.00 V to 0.00 V. Stock solutions of cadmium(II) and lead(II) were prepared, containing mercury nitrate, in order to spike the mine water sample, to verify the presence of these ions in the sample.

Gold ions present in the mine sample were concentrated by boiling the sample, on a low heat, to dryness. The remaining solid material was then redissolved in an acid mix similar to *aqua regia*; except that 3 part HNO_3 vs. 1 part HCl was preferred. The standard addition technique was employed in investigating the concentration of the gold ions present in the mine water sample. The constant volume, 2 ml, of treated mine water sample was spiked with increasing volumes of gold(III) stock solution (1.00×10^{-3} mol dm^{-3}) from 0.25 to 6 ml, whilst nitric acid (0.10 mol dm^{-3}) was utilised for a constant total volume of 15 ml.

The anodic stripping voltammetry for the standard addition trials on the mine water sample, in the absence of added mercury ions (since the mercury ions complicated the investigation), required a deposition potential of -0.35 V for a period of 30 seconds (stir-rate set at 3000 rev min^{-1}). The scan rate was set at 100 mV s^{-1} and the voltammograms were scanned to a final potential of 1.50 V.

2.3.2 The adsorptive cathodic stripping voltammetry studies:

The experimental procedure, for the adsorptive cathodic stripping voltammetric investigation of the mannan-gold interaction, required a deposition potential of 1.05 V for 250 seconds (stir-rate set at 3000 rev min^{-1}) on a glassy carbon electrode. The sample was stripped to a final potential of 0.00 V at a scan rate of 350 mV s^{-1}. A constant concentration of 0.30 μg l^{-1} mannan extract was maintained within the electrochemical cell during the AdCSV trials; testing the suitability of mannan as a ligand for gold(III) detection. The gold(III) concentration was varied to the detection limit of 6.20×10^{-8} mol dm^{-3}; utilising the linear sweep potential scan AdCSV in the presence of the mannan and absence of added mercury ions.

The AdCSV technique was then attempted on a glassy carbon electrode, in the absence the biological ligand, so as to ascertain the effectiveness of the mannan as a ligand for gold(III) detection. The parameters were set at a deposition potential, deposition time and final

potential of 1.05 V, 250 seconds (stir-rate set at 3000 rev min^{-1}) and -0.40 V respectively. The scan rate at which the stripping step was carried out was 350 mV s^{-1}. The initial experiments employed a gold(III) concentration ranging between 6.70×10^{-5} and 3.30×10^{-4} mol dm^{-3}; whilst later experiments, under the same conditions, were of the concentration order ranging from 7.00×10^{-7} to 3.00×10^{-4} mol dm^{-3}. The gold(III) concentration was obtained by adding between 8 µl and 3.5 ml volumes of gold(III) stock solution (1.00×10^{-3} mol dm^{-3}) to an appropriate volume of nitric acid (0.10 mol dm^{-3}), to achieve a total volume of 12 ml.

The platinum electrode was compared with the glassy carbon electrode, in the linear sweep potential scan AdCSV trials using mannan as the chosen ligand, for possible improved gold(III) detection properties. The platinum electrode was chosen despite the problem of peak reproducibility in gold(III) detection associated with this electrode (as mentioned in the introduction, section 1.3.4); since utilising the AdCSV technique may not present the problems associated with ASV and this electrode. Trials were completed in the absence of mercury(II). The deposition potential, deposition time and final potential of 1.05 V, 250 seconds and -0.40 V respectively, were applied to the platinum electrode system; containing a fixed concentration of mannan (0.3 µg l^{-1}). A scan rate of 350 mV s^{-1} was employed for this experimental procedure. The gold concentration, within the electrochemical cell, was varied in the range of 10^{-8} to 10^{-7} mol dm^{-3} by diluting appropriate volumes of gold solution (1.00×10^{-6} mol dm^{-3}) with nitirc acid (0.10 mol dm^{-3}) to obtain a total volume of 15 ml.

Experimental procedures were carried out to investigate the possible interference effects of silver(I) and copper(II) on the detection of gold(III); utilising the linear sweep potential scan adsorptive cathodic stripping voltammetry technique in the presence of the mannan ligand. The glassy carbon electrode was chosen as the working electrode, since noble metal electrodes (such as platinum) experience problems in peak reproducibility; as already mentioned in the introduction.[47, 48]

The copper(II) and silver(I) were in a ten-fold excess compared to the gold(III) concentration for these experiments. A 1 ml volume of gold(III) (1.00×10^{-3} mol dm^{-3}) was added to a 2 ml volume of silver(I) or copper(II) (1.00×10^{-3} mol dm^{-3}) and 12 ml of nitric acid (0.10 mol dm^{-3}) to give a total volume of 15 ml. The mannan concentration was kept constant at 0.24 μg l^{-1}. The parameters for the silver and copper interference studies were set at a deposition potential of 1.05 V for 200 seconds and potential range of 1.05 V to -0.60 V was scanned at a rate of 300 mV s^{-1}.

Adsorptive cathodic stripping voltammetry experiments could not be carried out on any of the other yeast cell wall samples, since the extracts were not water soluble. The remaining extracts (glucan, chitin and chitosan) were examined using the modified carbon paste electrode (MCPE) system. The behaviour of the mannan extract was also observed in the MCPE trials.

Trials utilising modified carbon paste electrodes, as the working electrodes, for the investigation of gold(III) were attempted with all the polysaccharide yeast cell wall extracts. The carbon paste electrode (CPE) was employed as the unmodified electrode for the gold(III) detection experiments. The modified carbon paste electrodes were prepared by mixing finely ground, carbon powder and dry biomass extract, followed by the addition of mineral oil to form a paste; which is then packed solidly into the glass tube. A polished copper wire was inserted into the mixture as a conductance point for each electrode prepared. The optimum ratio of the MCPE was found to contain 20 % biomass per weight, which is in agreement with the literature sourced.[64, 65] The carbon paste electrode was constructed in the same manner as the MCPE's ; except that the dry biomass extract was omitted.

The MCPE and CPE gold(III) detection systems were subjected to deposition potentials of 1.05 V for all the extracts. The deposition time and final potential of the experiments were set at 250 seconds (stir-rate of 3000 rev min^{-1}) and -0.40 V respectively. A scan rate of

350 mV s^{-1} was employed during the stripping process. The gold(III) concentration was varied within the 10^{-9} mol dm^{-3} range. Before each experiment was run, the electrodes required the removal of a small amount of paste, for the regeneration of the electrode surface.

A pH profile study was carried out on the mannan extract MCPE over a range of 0 - 11 pH units. The pH was adjusted using NaOH (0.50 mol dm^{-3}) (Saarchem Pty Ltd) standardised solution and nitric acid (0.10 mol dm^{-3}). For the pH study, a constant 100 µl volume of gold(III) (1.00×10^{-3} mol dm^{-3}) was diluted to a total volume of 10 ml with the appropriate volumes of NaOH and HNO_3.

Osteryoung and Barker square wave potential scan AdCSV were applied to the mannan MCPE experiments in an effort to lower the detection limit obtained by the linear sweep potential scan AdCSV. The square wave-forms had a step potential of 4 mV, a wave amplitude of 25 mV and a wave frequency of 15 Hz. The Barker square wave potential scan AdCSV had the deposition potential set at 1.05 V for 100 seconds and a final stripping potential of 0.00 V. The Osteryoung square wave potential scan AdCSV required the deposition potential set at 1.05 V for 250 seconds and a final stripping potential of 0.00 V. The gold(III) concentration was varied from 1.67×10^{-11} to 7.00×10^{-8} mol dm^{-3} by adding the appropriate volumes of gold(III) from the diluted stock solutions (1.00×10^{-8} and 1.00×10^{-6} mol dm^{-3} respectively). Nitric acid (0.10 mol dm^{-3}) was used to adjust the volumes of gold(III) added to a total volume of 16 ml.

2.3.3 Infrared Spectrophotometry:

In solid infrared analysis, the pressed disc technique was applied. Pure, dry potassium bromide (KBr) was intimately ground with a known weight of sample, using an agate mortar and pestle. The mixture was then added to a manually-operated press system for the preparation of a KBr disc.

The possibility of impurities in the KBr was eliminated with the use of a blank disc system. Care should, however, be taken to ensure both discs prepared are of equal thickness, otherwise inverse peaks may occur if the potassium bromide is damp or impure and this will be particularly noticeable if the reference disc is thicker than the sample disc.[68] Dry, analytical grade KBr is crucial to the IR experiment The dried potassium bromide was prepared by placing the KBr in a shallow dish in an oven, at 120 °C, for at least 24 hours. After sufficient drying time had passed, the KBr was stored in an oven at a temperature of ~ 100 °C.

Providing care has been taken in disc preparation, the final product should be slightly opaque, due to the presence of the sample (the blank disc should be transparent). Should the disc show a number of white spots, these would be a result of the mixture being unevenly ground. If the disc shows a tendency to flake, then excessive grinding of the powder is indicated. A disc changing to a cloudy colouring is indicative of water uptake by the disc and should be avoided.[68, 71]

The fully sealed IR cell, with calcium fluoride window, was chosen to investigate the solution phase gold-mannan interactions. A concentration exceeding 1 mg ml^{-1} of mannan was added to 1 ml of gold(III) stock solution (1.00×10^{-3} mol dm^{-3} in a 1 : 1 ratio of HCl and HNO_3) and thoroughly mixed. A suitable aliquot of liquid was then transferred into the fully sealed IR cell; such that no air bubbles could be detected between the calcium fluoride window plates.

2.3.4 Nuclear magnetic resonance spectometry:

Proton NMR sample preparation may often require a D_2O wash technique; particularly for complex polysaccharide molecules. The technique requires the dissolving of the sample in deuterated solvent followed by freeze-drying.[67] The procedure was repeated at least three

times to ensure that the water peak in the spectrum had been minimised and that complete exchange of the hydroxyl protons had occurred, in an effort to simplify the spectrum. The behaviour of the biological ligand under extreme acidic conditions was observed by the addition of 10 μl of concentrated nitric acid to the NMR sample tube and allowing the sample to equilibrate, before running the spectrum. Deuterated nitric acid was not used, as the effects of the acid on the sample was to be noted. The lowest concentration required to obtain a suitable proton NMR and COSY spectrum was 1 mg ml^{-1} mannan in D_2O solvent.

The NMR spectrum of a yeast or bacterial polysaccharide is complex as a result of signal overlapping, arising from the ring protons, and the presence of a water derived HOD (Hydrogen, Oxygen, Deuterium) signal, which may overlap other signals in the spectrum. However, the position of the HOD signal is temperature dependent and it is often possible to place the signal in a region of the spectrum where no other peaks occur, by altering the temperature of the probe.[70] The optimum temperature setting (30 °C) allowed for the observation of any peaks that may have fallen underneath the HOD signal.

NMR studies were employed to observe the proposed binding of the gold(III) to the biological extract. The set of experiments adhered to the same conditions as those applied to the biological sample. A 100 μl injection of gold solution (1.00×10^{-3} mol dm^{-3}) was added to the acidified biological extract in the NMR tube and allowed to equilibrate, at room temperature for 5 minutes, before an experimental run was attempted.

2.3.5 Computer modelling:

A Newton-Raphson energy minimisation of the mannan structure was performed in order to obtain an optimised structure of the conformer. Once the energy minimisation was complete, the metal, in the form of gold(III) chloride, was added to the system. The stoichiometry was assumed to be in a 1 : 1 ratio for simplicity and only a section of the polysaccharide-metal complex was modelled, as a representative of the whole molecule.

ELECTROCHEMICAL ANALYSIS OF GOLD(III):

Several methods are available for the determination of gold ions in low concentrations in various sample types. However, despite the wide variety of techniques available and the various modifications made to them, each of these techniques still lack at least one of the four (accuracy, precision, sensitivity and selectivity) requirements to complete the successful detection of gold ions as previously discussed in the introduction.

3.1. Anodic stripping voltammetry determination of gold(III):

Gold(III) may be easily reduced electrochemically, yet the determination of gold, requiring the deposition of gold onto a carbon or platinum electrode, is hampered by a slow nucleation process; as mentioned earlier in the introduction. The result of the slow nucleation process is poor accuracy levels in the quantification of gold because of different parts of the deposition time being consumed by the nucleation step.[50]

Anodic stripping voltammetry for the routine determination of gold ions in solution has been discouraged. The technique suffers from laborious preparation and conditioning of the electrodes, lengthy plating times, awkward sample-stripping solution changing procedures and the unsuitability of commonly used mercury electrodes (as a result of the mercury oxidation potential being considerable less positive than that of gold). However, some of these criticisms have often been levelled at the anodic stripping voltammetry technique in general.[51, 73]

Complexing agents added to the striping solution, in an attempt to overcome the problems associated with the use of the mercury electrodes and to lower the highly positive redox potentials observed in gold analysis, achieved little success. Activation of the electrode improved the slow nucleation problem; however, the activation led to an increase in the blank value and thus, a deterioration in the detection limit.[44, 50]

Huiliang *et al.*. proposed a co-deposition method of gold, on a glassy carbon electrode, with copper(II) and mercury(II) ions in solution, if not already present in the sample, followed by pulsed stripping in the presence of a stripping solution.[44] The result was an accurate, reasonably sensitive method, with a detection limit of 3 $\mu g\ l^{-1}$. However, the procedure required long deposition times, in excess of 900 seconds, and complications may arise if this method is applied to a more complex sample, such as a mining effluent.

Anodic stripping voltammetry trials for the detection of gold(III), employing the glassy carbon electrode with a known concentration of gold(III) in solution, were attempted in this work. However, the stripping peak position, area and width all varied considerably using this method. The co-deposition/amalgam formation of gold ions in the presence of mercury(II) was then explored. A deposition potential of 0.10 V was chosen to avoid the mercury film formation on the electrode surface. The conventional linear stripping technique was employed in place of pulsed methods and a separate stripping solution proposed by Huiliang *et al.*.[44]

The presence of mercury(II) enhanced the sensitivity, accuracy and reliability compared with the glassy carbon electrode in the absence of mercury(II). The glassy carbon electrode experiments (without mercury ions) resulted in broad peaks, with a large degree of unreliability in peak size and position. The experiment was carried out in a variety of electrolyte solutions including nitric, hydrochloric and perchloric acids (all 0.10 mol dm^{-3}) . The acids were used to make up the sample of gold stock solution to the required volume. Combinations of acids were also attempted, such as the 50 / 50 mix of 0.10 mol dm^{-3} nitric and hydrochloric acids and the 50 / 50 mix of 0.10 mol dm^{-3} nitric and perchloric acids. In the presence of acid mixtures as proposed electrolytes, the peak attributed to the gold(III) presence began to disappear with time.

Upon closer investigation of the individual acids utilised, perchloric acid gave the highest peak currents. However, after two consecutive runs on the sample, the observed peak currents decreased considerably in intensity and shifted in position. Hydrochloric acid resulted in current peaks with uncertain positions and unreliable current intensities. Literature suggests the reason for this is the important role the chloride ions play in the stabilisation of gold(III/I) interactions and the formation of gold chloride intermediates, which would hamper the detection of gold(III) in solution. The choice of electrolyte is crucial to the success of the experiment.[38, 74] Nitric acid was found to be the most suitable electrolyte for gold(III) analysis. Figure 3.1 illustrates the increase in the peak current observed as the concentration of gold(III) (in nitric acid) is increased.

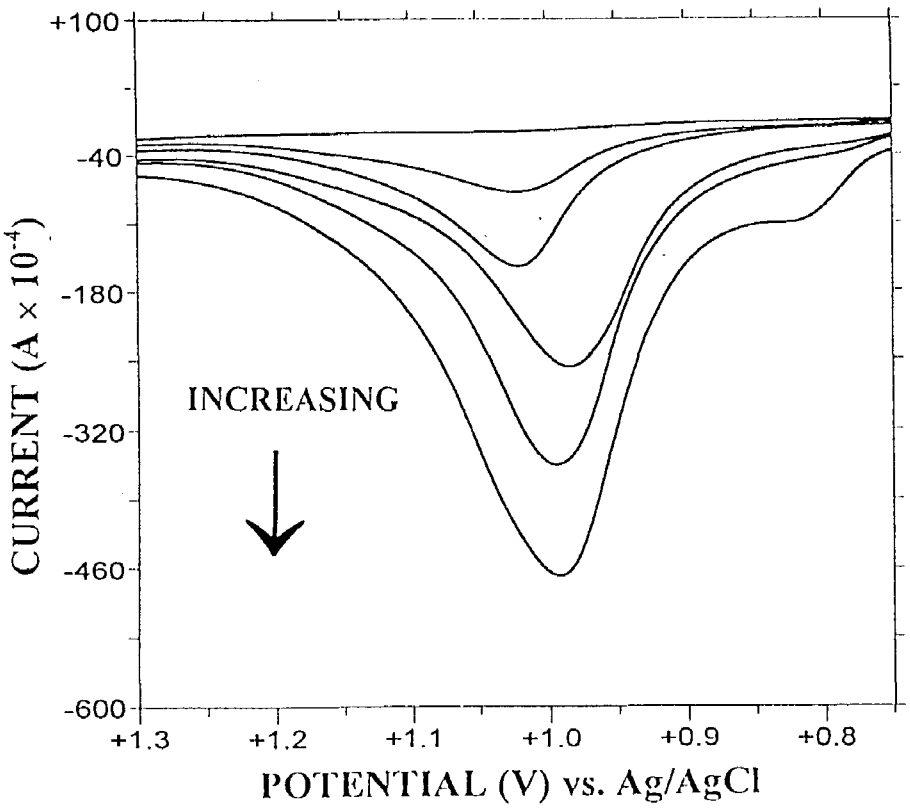

Figure 3.1: The anodic stripping voltammetry determination of gold(III) in the presence of mercury ions (1.50×10^{-3} mol dm^{-3}). Deposition potential = 0.10 V Deposition time = 100 seconds Scan rate = 100 mV s^{-1}.

The gold(III) concentration of each current peak was 6.70×10^{-7} mol dm^{-3}, 1.30×10^{-6} mol dm^{-3}, 2.00×10^{-6} mol dm^{-3}, 2.70×10^{-6} mol dm^{-3}, 3.30×10^{-6} mol dm^{-3} and 4.00×10^{-6} mol dm^{-3}, respectively (in increasing order). The anodic stripping peak increased with the increase of gold(III) ion concentration, in the nitric acid and mercury containing sample solution. An acceptable deviation of less than 5 %, for the current peak, was achieved.

A slight shift in peak position was observed for the two peaks of lowest gold(III) concentration (1.02 V) compared with the remaining peaks observed at 1.00 V. The reduction of gold(III) chloride has been observed in the region of 1.00 V versus the saturated calomel electrode.[75] The slight shift in peak position of 0.02 V may be attributed to gold nucleation effects on the glassy carbon electrode (see section 1.3.4) and to the differences in gold(III) concentration; the effects of which are explained by the Nernst equation (see Equation (1.3) in the introduction).

3.2 Application of anodic stripping voltammetry to a mining sample:

3.2.1 Determination of interfering ions present in the sample:

The major advantage of electrochemistry, as a detection tool in trace metal analysis, is the ability of the system to simultaneously detect the presence of more than one metal ion.[30, 31] The ASV technique was applied to the Deelskraal mine water sample for the detection of any metal ions present in solution. In the negative potential window, two stripping current peaks were observed, for the mine water sample, at -0.41 V and -0.61 V (see Figure 3.2(a))

The mine water sample was then spiked with lead(II) and cadmium(II) and the magnitude of the current peaks increased upon increasing the concentration of the respective ions; as illustrated in Figure 3.2(b) The current peaks observed at -0.41 V and -0.61 V were thus attributed to lead(II) and cadmium(II) respectively, present in the mine water sample.

A 'blank' solution sample (*i.e.* in the absence of the mine water sample) was run in order to minimise the effect of any common ions present in water; that may also be present in the mine water sample.

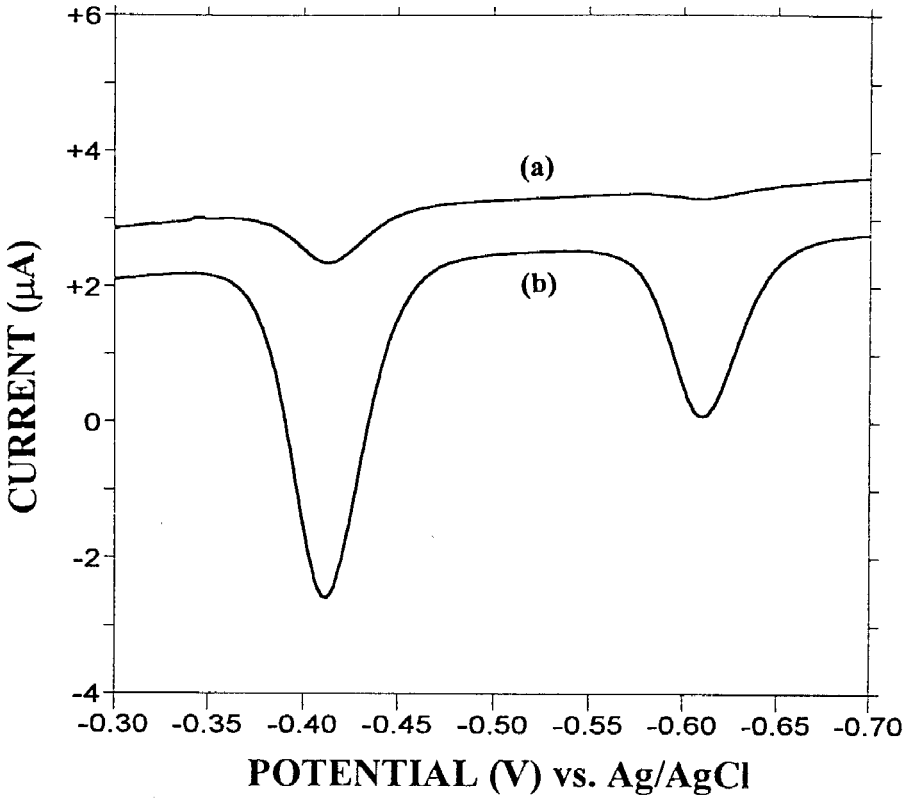

Figure 3.2 The ASV current peaks observed in the mine water sample (a) and on addition of lead(II) and cadmium(II) to the sample (b) Deposition potential = -1.00 V Scan rate = 100 mV s^{-1} Deposition time = 300 s

The concentrations of the cadmium and lead ions present (15 μg l^{-1} and 76 μg l^{-1} respectively), were found to be within the acceptable limits for drinking water in South Africa, which has a maximum limit of 20 μg l^{-1} for cadmium and 100 μg l^{-1} for lead.[11] Atomic absorption spectrometry, performed independently of this study, completed on the Deelskraal mine sample proposed the presence of gold ions only, however, the oxidation state of the gold could not be verified utilising this method.

3.2.2 Electrochemical investigation of gold in the mine sample:

Electrochemical detection of metal ion speciation is unique for that ion and its oxidation number, since there is only one assigned redox peak potential for a redox couple. Experimental parameters may be optimised so as to avoid the interference of other metal ions present in the solution.[30, 31, 49] The initial deposition potential was set at -0.35 V, to avoid the effects of the cadmium and lead ions now known to be present in the mine water sample. The standard addition technique was attempted on the Deelskraal sample by keeping the amount of unknown sample (2 ml in a 15 ml total volume sample) constant in solution and gradually increasing the gold(III) ion concentrations from 1.67×10^{-5} mol dm^{-3}, to 3.10×10^{-4} mol dm^{-3} to 3.70×10^{-4} mol dm^{-3} and 4.30×10^{-4} mol dm^{-3}; seen in Figure 3.3.

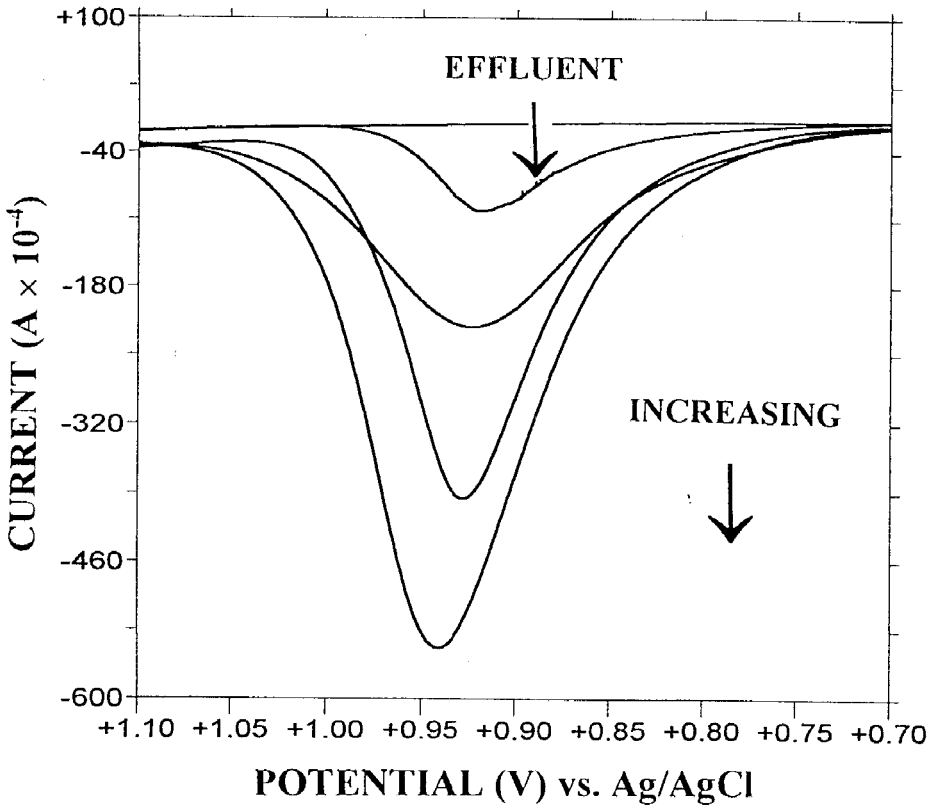

Figure 3.3: Anodic stripping voltammetry determination of gold(III) in a pre-concentrated mine water sample, in the absence of mercury ions.

Scan rate = 100 mV s^{-1} Deposition potential = -0.35 V

An increase in the gold(III) concentration would result in an increase in the reduction peak, if the reduction was in fact due to the reduction of gold(III) to gold(0). The glassy carbon electrode was chosen as the working electrode and mercury ions were omitted; since mercury was found not to be suitable for gold ion detection in a complex sample. In an attempt to concentrate the gold(III) ions present in solution, the sample was evaporated to dryness and then the residue was redissolved in a form of "reversed *aqua regia*"; where a 1 : 3 ratio of HCl to HNO_3 was preferred.

The stripping peak responsible for the transition of gold(III) to gold(0) was observed at 0.93 V in the mine water sample. The observed peaks for the reduction of gold(III) to gold(0) has been cited in this region; the reduction of $AuCl_4^-$, for example, occurs at a potential of 1.00 V vs. the Ag / AgCl reference electrode in acidic media.[38, 75] The slightly lower potential observed for the mining sample compared with the literature value, may be the result of different conditions that prevailed.

The gold ions present in the mining sample were discovered to be in the gold(III) oxidation state and the concentration of the ions present was calculated, using established methods, to be in the order of 2.53×10^{-5}mol dm^{-3}. However, the possibility exists that the gold present in the sample was actually in the gold(0) state and that addition of the *aqua regia* acid form forced the dissolution of the metal into the ion form; characteristically the gold(III) oxidation state. The assumption is based on the fact that in the industrial crushing process water is used, no other chemical is added until later, and it is unlikely that metallic gold from the ore will naturally go into solution in the water. Fine sand-like grains were also observed in the mining sample, which appeared to contain elemental gold.

The application of anodic stripping voltammetry has proven successful, under the defined experimental parameters, in the determination of gold ions in a mine water sample. Lower detection limits for gold in more complex matrices, such as the presence of gold drugs in biological tissue, may now be attempted utilising the ASV application.

THE INTERACTION OF GOLD(III) WITH THE YEAST CELL WALL EXTRACTS:*

4.1 Interaction of gold(III) with mannan:

4.1.1 Glassy carbon electrode studies:

Adsorptive cathodic stripping voltammetry in the presence of mannan:

The recovery of gold from dilute aqueous solutions by biomass has been investigated as an alternative to the carbon-in-pulp technology, currently used in the gold industry. Most types of biomass bind gold to some extent when exposed to a solution containing either gold(I) (usually as $[AuCN)_2]^-$) or gold(III) (in the form of $AuCl_4^-$). However, the binding of the metal ion to the biomass does not follow a set pattern and the uptake of the gold ions is dependent on the micro-organism, species and experimental conditions.[76]

Mannan, a chief constituent of the yeast cell wall, is water soluble and readily dissolves in the triply distilled deionised water and is ideally suitable for the electrochemical detection of a possible mannan-gold complex. Differential pulse voltammetry (DPV) has been widely used for the determination of metal ions in solution, however, attempts to perform stripping studies with pulsed potential scans have, in some cases, resulted in signals that were more complex than those observed for the linear sweep potential scan techniques.[77]

The development of adsorptive stripping voltammetry (AdSV) has, on the other hand, allowed for the determination of many trace metals; including those that could not be determined by conventional anodic stripping voltammetry (ASV). Apart from extending the

* This work has been accepted for publication and is not further referenced in this chapter. Lack B., Duncan J. and Nyokong T., Adsorptive cathodic stripping voltammetric determination of gold(III) in the presence of yeast mannan, accepted October 1998, Anal. Chim. Acta, in press.

scope of ASV, AdSV offers improved schemes for determination of metals, which suffer from difficulties such as extreme redox potentials or poor selectivity.[52, 78]

Trials were completed utilising the adsorptive cathodic stripping voltammetry (AdCSV) method, with mannan as the chosen ligand, in the absence of mercury(II) ions. The successful fruition of the AdCSV technique relies on the formation of a metal-ligand complex, which will undergo controlled interfacial accumulation of the complex onto the electrode during the deposition step.

Ligands suitable for AdCSV must thus be selective for the metal of interest and the complex formed must be able to adsorb to the electrode surface. The extent of complex adsorption has been related to the solubility of the complex; strong adsorption being observed for complexes that exhibit low solubilities.[52] Figure 4.1 shows the AdCSV of gold(III) (1.00×10^{-4} mol dm^{-3}) in the absence (Figure 4.1(a)) and the presence (Figure 4.1(b)) of yeast mannan (0.30 $\mu g\ l^{-1}$).

Addition of mannan resulted in the enhancement of the stripping peak of gold(III), indicative of the *in situ* formation of the gold-mannan complex; as seen in Figure 4.1. The voltammetric response of the surface confined species is directly related to their surface concentration and at low adsorbate concentrations, the surface concentration is directly proportional to the concentration in the bulk solution.[79]

Figure 4.1(a) shows that two reduction peaks were observed for gold(III), in the absence of mannan, under the experimental conditions chosen. The second peak was observed around -0.09V and was considerably smaller in magnitude, compared with the first peak at 0.29 V. Two reduction peaks for the reduction of gold(III) on carbon electrodes have been reported.[80] A two step reduction of gold(III), via gold(I), is proposed under the experimental conditions; leading to the two reduction peaks observed in Figure 4.1(a). Gold(III) is readily reduced to gold(I) at pH values greater than 2 pH units.[45]

The adsorptive cathodic stripping voltammetry of gold(I), presumed to be formed when the pH of gold(III) solutions was adjusted to values greater than 2 pH units, gave a reduction peak at -0.02 V; a potential close to that associated with the proposed reduction of gold(I) in Figure 4.1(a).

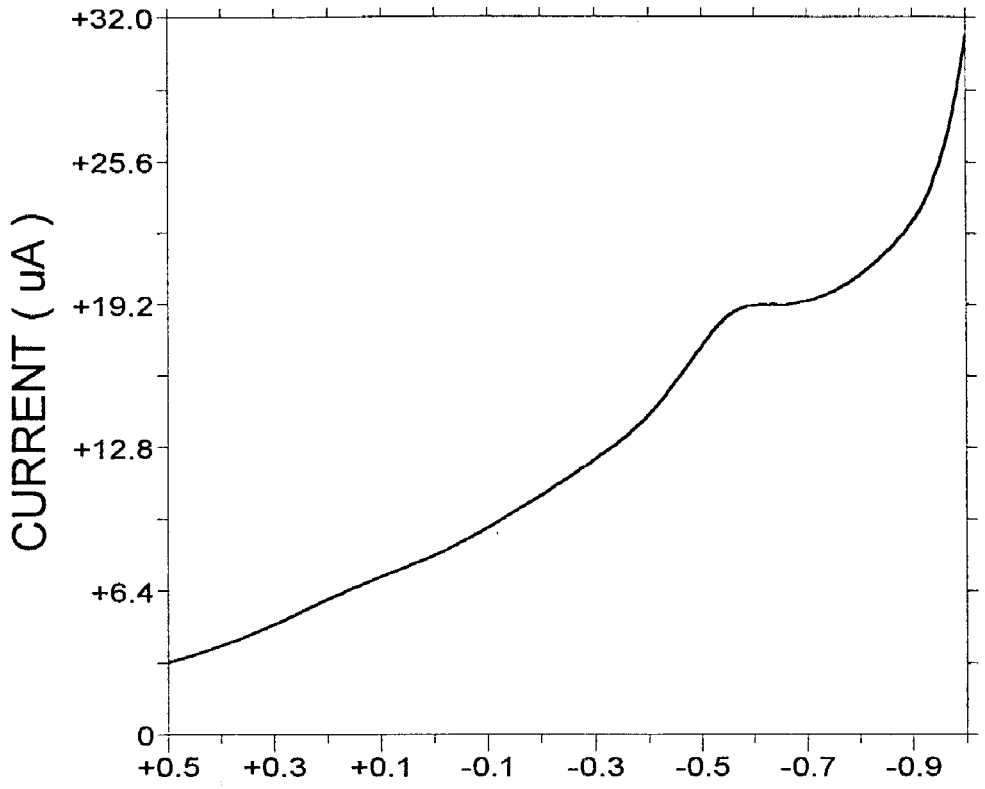

Figure 4.1 The adsorptive cathodic stripping voltammograms obtained for gold(III) at a concentration of 1.00 $\times 10^{-4}$mol dm^{-3}on a GCE in the absence (a) and presence (b) of 0.30 μg l^{-1}of yeast mannan. The scan rate = 100 mV s^{-1}.

The peak height for the second reduction at -0.09 V, Figure 4.1(a), is smaller than that for the first reduction; implying that a fewer number of electrons are involved in the former. The more positive peak at 0.29 V is suggested to be the result of the gold(III) reduction, whilst the peak at -0.09 V is assigned to the gold(I) reduction. In the presence of mannan the voltammetric peak observed at 0.29 V shifted to 0.33 V. The magnitude of the shift in the metal reduction peak, on addition of the ligand, is indicative of the stability of the metal-ligand complex. A positive shift of 0.04 V, for the gold(III) reduction peak on the addition

of mannan, shows that the resulting mannan-gold complex is more readily reduced than the gold(III). However, a shift of 0.04 V is comparatively small for complex formation reactions, which suggests that the gold-mannan complex is not very stable.

The peak observed near -0.09 V, which is associated with the gold(I) reduction was also enhanced on addition of mannan to the gold(III) solution. In the presence of mannan, the gold(I) reduction peak shifted to a more negative potential, -0.15 V, showing that the resulting complex is less readily reduced than the gold(I) species. The measure of the potential shift for the second peak is -0.06 V, which is slightly larger than the shift observed for the first peak associated with the formation of a gold(III)-mannan complex. The larger shift value offers the possibility that the gold(I)-mannan complex is slightly more stable than the gold(III)-mannan complex. The adsorptive cathodic stripping voltammogram of yeast mannan alone, in the absence of gold(III), was observed at -0.55 V (Figure 4.1(c)).

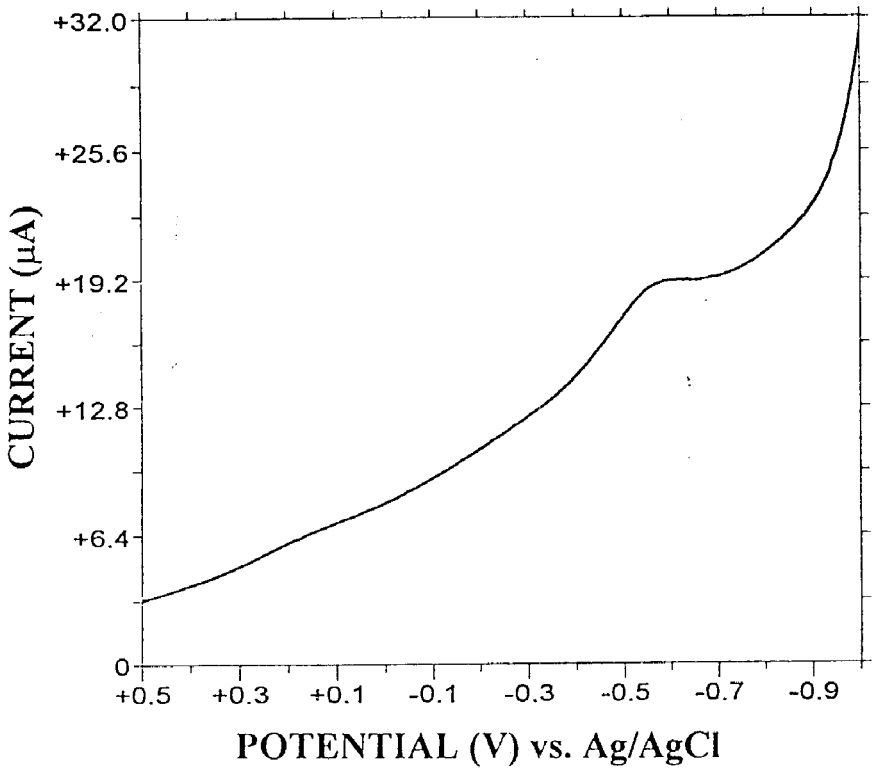

Figure 4.1(c): The AdCSV of 0.11 μg l^{-1} in the absence of gold(III).

Scan rate = 100 mV s^{-1} Deposition potential = 1.05 V

In the presence of mannan, the voltammetric peak observed for gold(III) at 0.29 V shifted to 0.33 V; see Figure 4.1(a) and (b). The stripping current peak for the proposed metal-ligand complex reduction, is close to the observed peak for the metal reduction alone; hence, the reduction of the complex may be attributed to reduction occurring at the metal site.

Various parameters were studied in an attempt to optimise the conditions for the detection of the gold-mannan complexes formed. The different parameters studied include those affecting the formation of the metal-ligand complex, its adsorption onto the electrode and the parameters responsible for the stripping of the complex from the electrode surface. Figure 4.2 illustrates the variation of the AdCSV currents for the peak, assigned to the reduction of gold(III) in the presence of mannan, as a function of the time used for the deposition (accumulation) of the gold(III)-mannan complex onto the electrode surface.

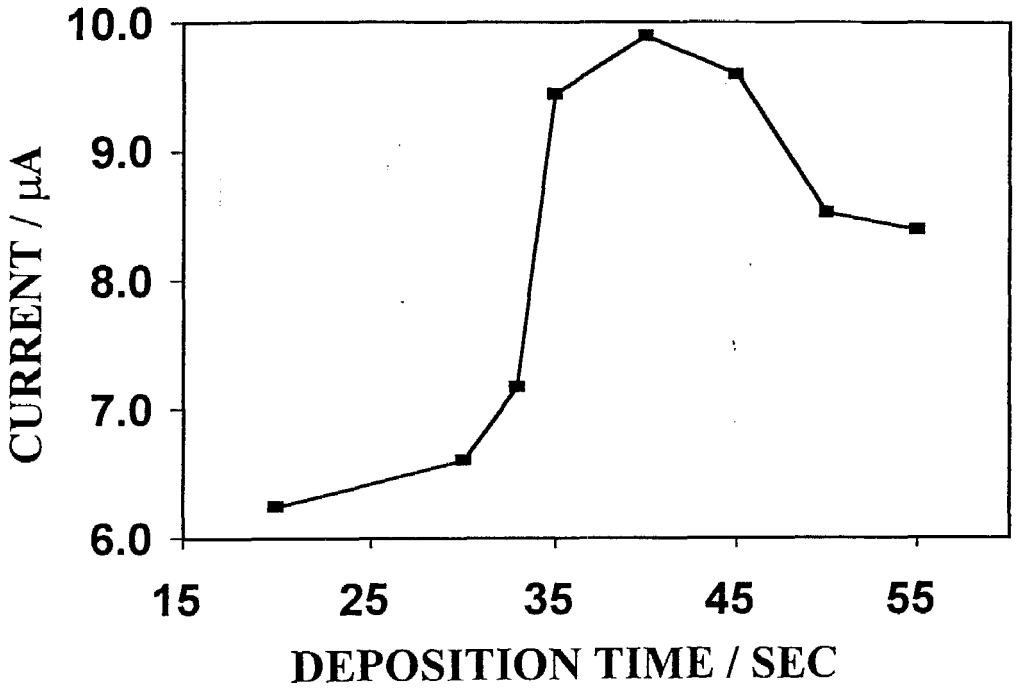

Figure 4.2: The dependence of the AdCSV peak currents on the deposition time for gold(III) at a concentration of 1.00×10^{-4} mol dm^{-3} in the presence of 0.30 μg l^{-1} mannan. Scan rate = 100 mV s^{-1} Deposition potential = 1.05 V vs. the Ag/AgCl

In Figure 4.2, a rapid increase in the cathodic stripping current with deposition time was observed. The dependence of the peak current on the deposition time is limited by the saturation of the electrode surface in an adsorptive process; resulting in a peak of the currents for the plot of the deposition time versus current. The maximum current value occurred at a deposition time of 40 seconds and hence this time was used for all AdCSV studies.

The potential required for the deposition of the gold(III)-mannan complex onto the electrode, the deposition or accumulation potential, varied with the adsorptive cathodic stripping voltammetry currents, as shown in Figure 4.3. The optimum deposition potential of 1.05 V was employed throughout further AdCSV studies.

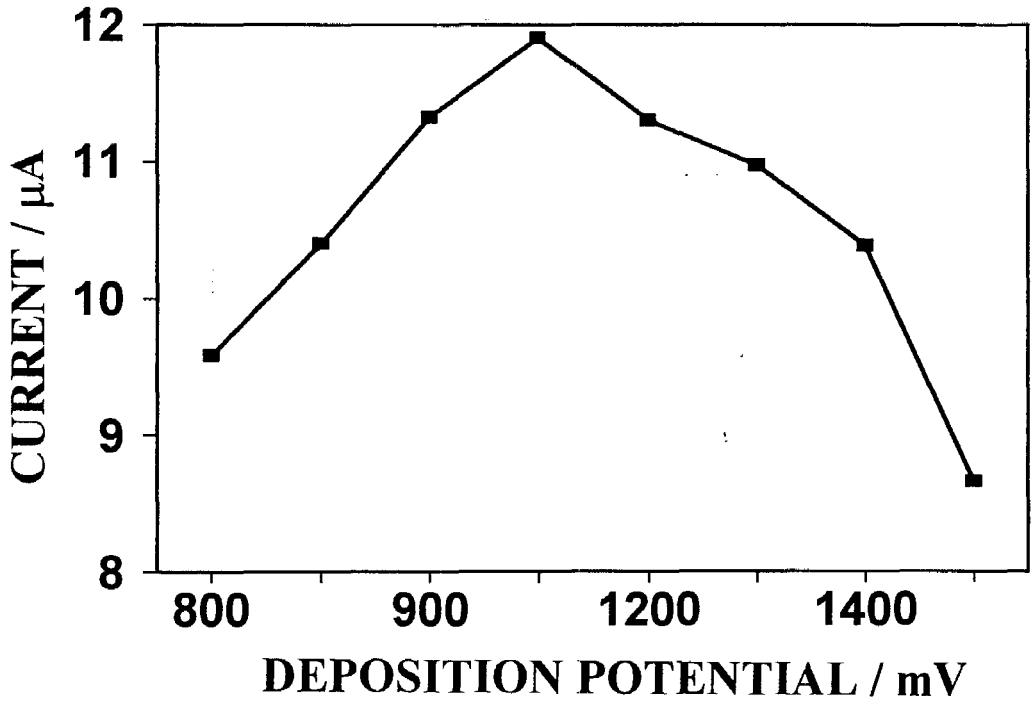

Figure 4.3: The dependence of the AdCSV peak currents on the deposition potential for gold(III) (1.00×10^{-4} mol dm^{-3}) in the presence of 0.30 μg l^{-1} of mannan. Scan rate = 100 mV s^{-1} Deposition time = 250 seconds

The AdCSV currents increased with an increase in the concentration at low mannan concentration levels; as seen in Figure 4.4. At high mannan concentrations the current response decreased as the concentration of the mannan increased. The observation implies that the activity of the electrode surface decreases at high concentrations of the ligand. The reason for the decrease in electrode activity may be the result of full coverage of the electrode surface and/or the possibility of the ligand occupying free sites on the electrode and competing with the metal-mannan complex for electrode space.

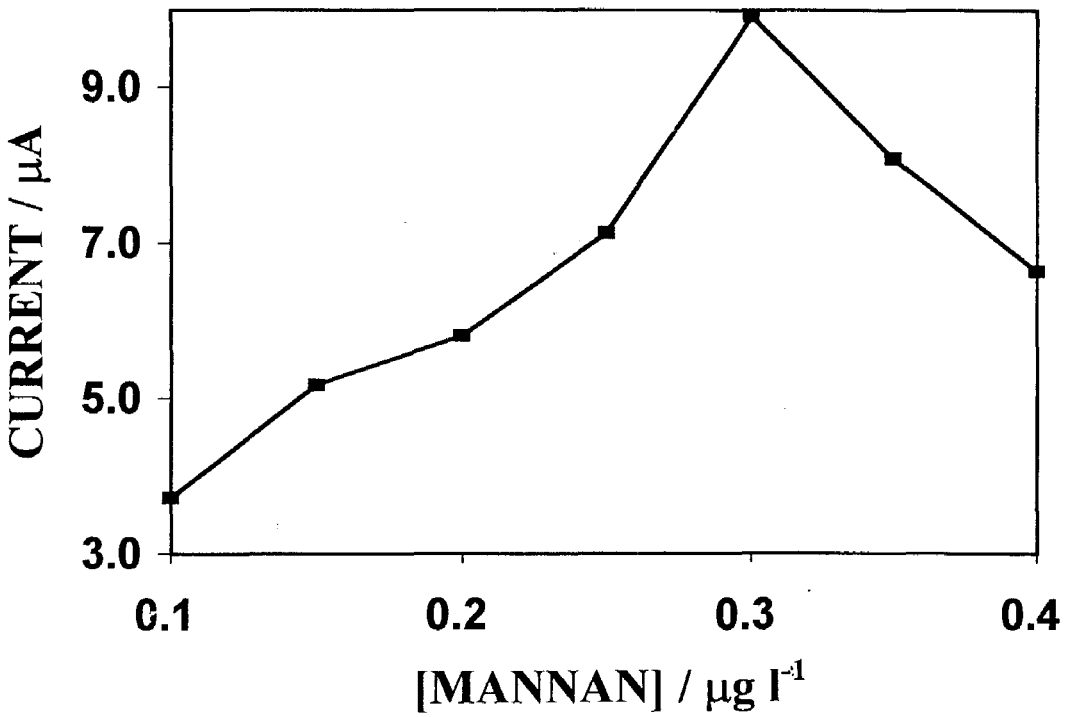

Figure 4.4: The influence of the yeast mannan concentration on the AdCSV peak currents for 1.00×10^{-4} mol dm^{-3} gold(III). Scan rate = 100 mV s^{-1}

A linear variation of the AdCSV peak currents, of the peak assigned to the gold(III)-mannan complex with changes in the gold(III) concentration, was obtained under optimal experimental conditions and is illustrated in Figure 4.5. The mannan concentration was kept

constant at 0.30 μg l^{-1}. The range applied for the gold(III) concentration variation was from 7.00×10^{-7} to 3.00×10^{-4} mol dm^{-3}.

A regression equation of y = 1.7x + 5.6 (y = peak height and x = concentration) and a correlation coefficient of 0.995 were obtained for the linear plot in Figure 4.5. The relative standard deviation, obtained under optimal experimental conditions for the gold(III) detection (1.00×10^{-4} mol dm^{-3}), was 3 %. The detection limit for the determination of gold(III) under the assigned experimental conditions was found to be 9.00×10^{-9}mol dm^{-3}.

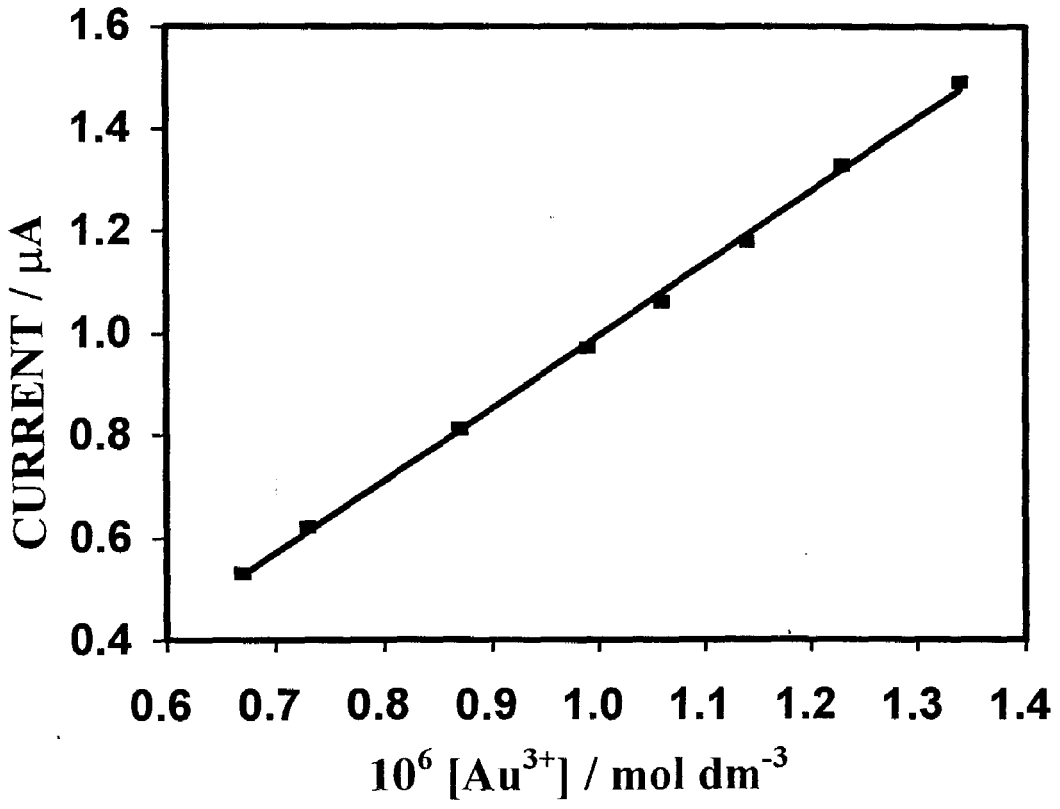

Figure 4.5: The variation of the concentration of gold(III) with the adsorptive cathodic stripping currents in the presence of 0.30 μg l^{-1} yeast mannan. Scan rate = 100 mV s^{-1} Deposition potential = 1.05 V Deposition time = 40 s

The proposed mechanism for the AdCSV of the gold(III) mannan interaction is as follows:

Au^{3+} + Man ↔ [AuMan] (in solution; prior to accumulation potential applied)

[AuMan] ↔ $[AuMan]_{ads}$ (adsorption onto electrode during applied potential

$[AuMan]_{ads}$ + $3e^-$ ↔ Au^0 (stripping step)

Adsorptive cathodic stripping voltammetry of gold(III) in the absence of mannan:

The reduction of gold(III), however, on unmodified carbon electrodes characteristically exhibited a single reduction peak associated with the three-electron reduction of gold(III) to gold(0).[46] However, there have been reports of two reduction peaks being observed on glassy carbon electrodes in particular, as mentioned earlier in section 4.1.1.[80] The peak has been shown to be highly dependent on the nature of the electrode, the electrolyte and experimental parameters chosen and the presence of any complexing ions.[44, 46, 51, 63, 77, 80, 81] A 'blank' sample (*i.e.* gold(III) in the absence of the mannan ligand), primarily to ascertain the suitability of mannan as a ligand for gold(III) detection, was run for each of the gold(III) concentrations used in the AdCSV experiments in the presence of mannan.

In the absence of mannan and mercury ions and with a gold(III) concentration of 1.00×10^{-4} mol dm^{-3}, as seen in Figure 4.1(a), two stripping peaks were observed at 0.29 V and -0.09 V. The first peak height is larger than the second and is considered to be the result of the gold(III) to gold(0) reduction. The AdCSV peaks observed for gold(III) solutions are proposed to be the result of gold(III)-chloride complex present.

Concentrations of gold(III) lower than 1.00×10^{-5} mol dm^{-3} resulted in a single current peak at 0.38 V being observed for the AdCSV of gold(III) in the absence of mannan; Figure 4.6. The concentration of the gold(III) observed in Figure 4.6, is 1.30×10^{-7} mol dm^{-3}. The absence of the second peak, assigned to the gold(I) reduction complex intermediate, is thought to be concentration related; since even in the presence of mannan at the same

gold(III) concentration, the second peak was also notably absent. The peak assigned to the gold(III) to gold(0) reduction could be observed at concentrations in the order of 9.40×10^{-8} mol dm^{-3}; thus the potential at which the gold(III) is observed, is also concentration dependent.

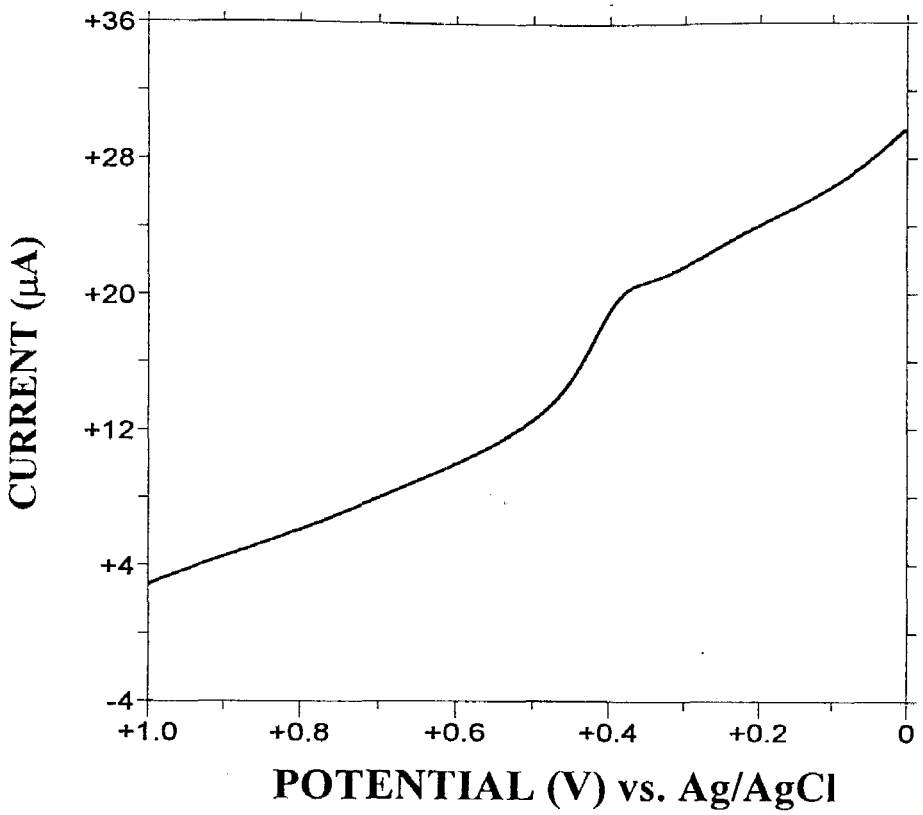

Figure 4.6: The AdCSV peak for the reduction of gold(III) (1.30×10^{-7} mol dm^{-3}) in the absence of mannan and mercury ions. Scan rate = 350 mV s^{-1}

A linear variation of the assigned gold(III) reduction peak current with concentration, ranging from between 6.00×10^{-7} to 1.40×10^{-6} mol dm^{-3}, is illustrated in Figure 4.7 and a regression value of 0.989 was calculated from the data.

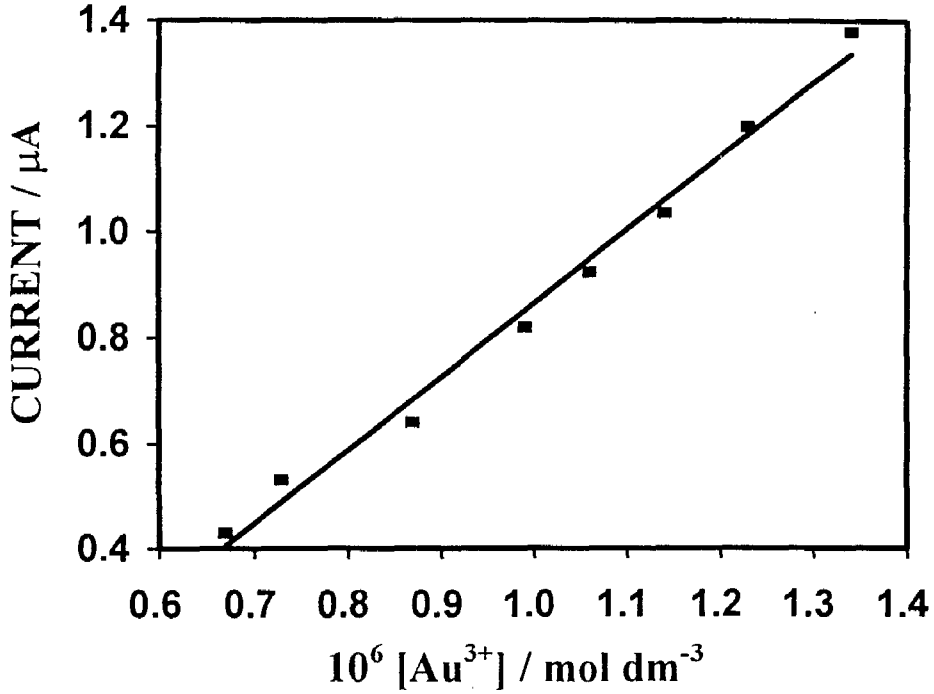

Figure 4.7: Variance of AdCSV currents with increasing gold(III) concentration. Deposition potential = 1.05 V Scan rate = 350 mV s^{-1}

Scanning electron microscopy of the AdCSV gold-mannan complex:

Scanning electron microscopy represents another widely used technique available for obtaining *ex-situ* information on the surface morphology, and in certain cases the chemical composition, of a sample. The scanning electron microscopy process utilises the secondary electrons emitted for detection purposes as the electron beam is scanned across the sample surface[31] The scanning electron micrograph (SEM) was employed to verify the electrochemical data on the proposed gold(III)-mannan complex formation.

Figure 4.8, the scanning electron micrograph, illustrates the gold(III)-mannan complex observed on a glassy carbon electrode surface, as a result of the adsorptive cathodic mode of stripping voltammetry. The 'fern-shaped', crystalline substance is the biological extract

mannan; whilst the small, ball-like structures are the result of the metal present. A metal-ligand interaction occurs at the sites on the SEM where both the metal and mannan are visibly attached to each other. The SEM were obtained, after the optimised experimental conditions had been established, at a magnification of × 5000.

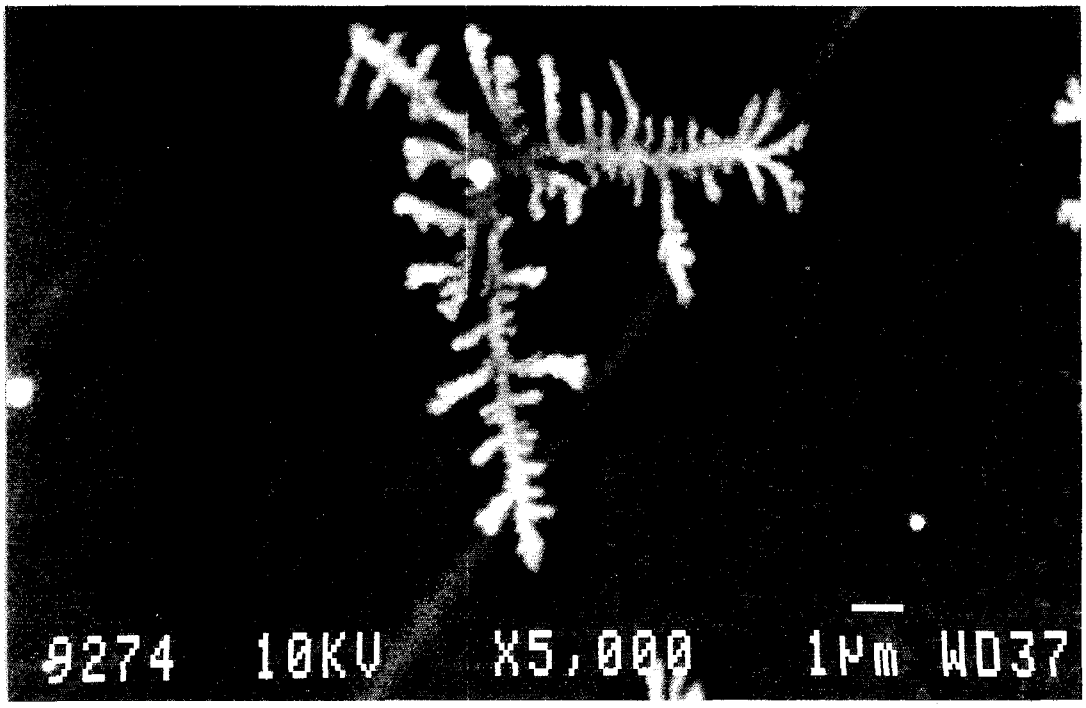

Figure 4.8: The scanning electron micrograph (× 5000) of the GCE electrode surface in the presence of gold(III) (1.00×10^{-5} mol dm^{-3}) and mannan (0.30 μg l^{-1}).

4.1.2 Platinum electrode studies:

The use of a platinum electrode has been proposed for the investigation of gold(III) in solution.[44, 77] In this study, AdCSV, in the presence of mannan, was attempted with the platinum electrode as the working electrode of choice. Noble metal electrodes have, however, been shown to lack reproducibility; both quantitatively and qualitatively. The lack

of reproducibility of the current peaks has been attributed to oxide film formation on the electrode surface in oxidising and non-complexing media. The platinum metal electrode in particular, will be more likely to fall prey to chloride film formation, at the electrode surface, in the presence of any hydrochloric acid.[47]

Figure 4.9 illustrates the AdCSV gold(III) reduction peak in the absence (a) and presence (b) of mannan. The addition of mannan resulted in the enhancement of the gold(III) stripping peak and a shift in the reduction peak position from 0.31 V to 0.37 V; indicative of the *in situ* formation of a gold-mannan complex.

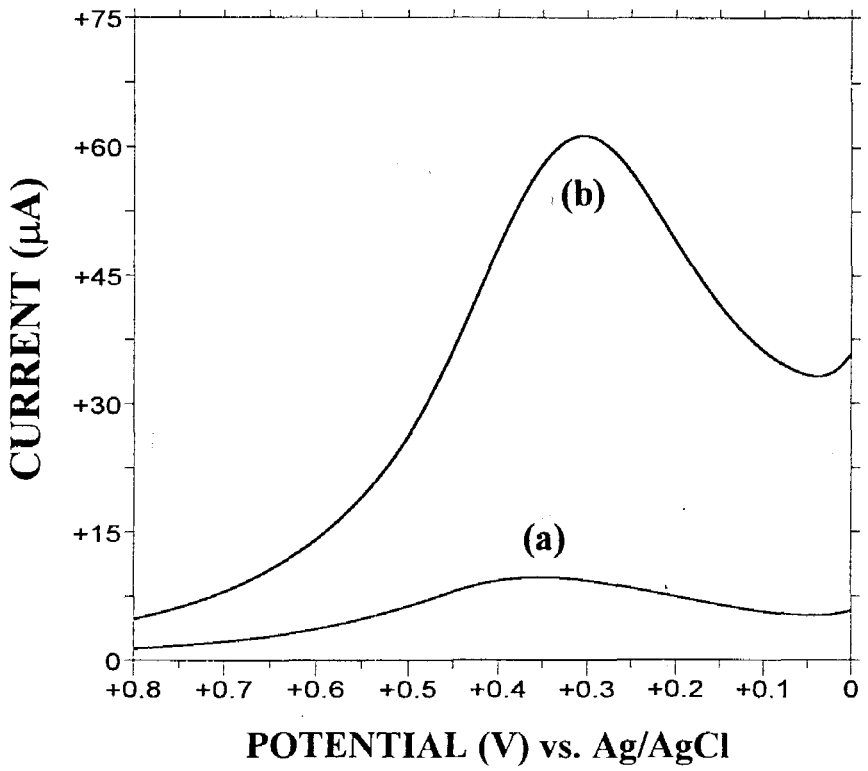

Figure 4.9: The AdCSV on a platinum electrode in the (a) absence and (b) presence of mannan for gold(III) (1.30×10^{-7} mol dm^{-3}) detection. Deposition potential = 1.05 V Deposition time = 250 seconds Scan rate = 350 mV s^{-1}

A negative shift of 0.06 V for the gold(III) reduction peak on the addition of mannan, shows the resulting gold-mannan complex is less easily reduced than the gold(III) alone. The shift of 0.06 V is relatively small, indicating that the complex formed between gold(III) and mannan is not very stable. The platinum working electrode did not exhibit a second current peak, assumed to be the result of a gold(I) intermediate complex, as was the case for the glassy carbon electrode.

The advantages of the platinum electrode over the glassy carbon electrode included a lower background current and the peak obtained for the gold(III) reduction could be obtained at considerably shorter deposition times on a smaller diameter electrode surface. Bond *et al.* have recently (1997) compared the reduction of gold(III) on various working electrodes and the platinum electrode was found to be the optimum electrode.[77]

The platinum electrode thus, appears an ideal electrode for the detection of gold(III) in solution, however, an experimentally observed major drawback of the electrode prevents the furthered use of this electrode. The disadvantage is in the form of peak reliability and the problems associated with electrode cleaning. Gold(III) appears to interact strongly with the platinum electrode surface, particularly in the absence of mannan, and with further experimental runs, the peak currents were not reproducible.

Several mechanical and electrochemical cleaning methods were attempted to improve the reproducibility of the peak, including soaking the platinum electrode in concentrated hydrogen peroxide followed by electrochemical cleaning and furthered polishing on a Buehler felt pad; however, only slight improvements were noted. The interference of platinum ions on the determination of gold(III) in solution has been observed.[50] Similar interactions may be taking place at the electrode surface, under the harsh acidic conditions of the experiment. However, since the gold(III) stock solution (1.00×10^{-3} mol dm^{-3}) contained a 50 : 50 mixture of hydrochloric and nitric acid, the chloride film formation was the probable cause of the deviations observed in the gold(III) analysis.

4.2 Interferences of copper(II) and silver(I) on the gold-mannan complex formation:

Interferences for the adsorptive cathodic stripping voltammetric determination of the gold(III) may be due to peak overlap or to the formation of inter-metallic compounds. In the presence of complexing ligands, such as mannan, interferences may be the result of competition of the metal ions for the ligand binding sites provided. Many metal ions have only a slight effect on the anodic stripping voltammetry determination of gold(III); even when present in a large excess (120-fold) with respect to gold.[81]

However, silver is known to interfere significantly with gold determination; a ten-fold excess of silver(I) can seriously obscure the stripping voltammogram of gold. Copper displays a stripping peak several hundred millivolts more cathodic than the gold peak; however, gold and copper are known to form inter-metallic compounds.[44] Studies have shown that the yeast biomass from *S. cerevisiae* preferentially accumulates copper ions over cadmium and cobalt ions.[82]

Copper uptake capacities range from 0.05 to 0.184 mmol g^{-1} dry weight, whilst values of 0.034 to 0.193 mmol silver g^{-1} dry weight biomass have been reported for the yeast, *S. cerevisiae*.[83] The gold(III)-mannan complex formation may be affected by the presence of these interfering ions and studies were carried out to ascertain the effects. Figure 4.10 shows the AdCSV of gold(III) (6.00×10^{-5} mol dm^{-3}) and copper (II) (1.20×10^{-4} mol dm^{-3}) in the absence (Figure 4.10(a)) and presence (Figure 4.10(b)) of mannan (0.24 μg l^{-1}).

The gold(III)-mannan complex AdCSV peak was observed at 0.33 V when only gold(III) and mannan were present in solution; as seen in Figure 4.1(b). The addition of copper(II) to gold(III) and mannan, seen in Figure 4.10(b), did not change the peak position significantly compared with the observed peak in Figure 4.1(b) However, a large increase in the background current of the adsorptive cathodic stripping voltammograms in the

presence of copper(II) was noted, hence the peak associated with gold(I) was not observed in Figure 4.10.

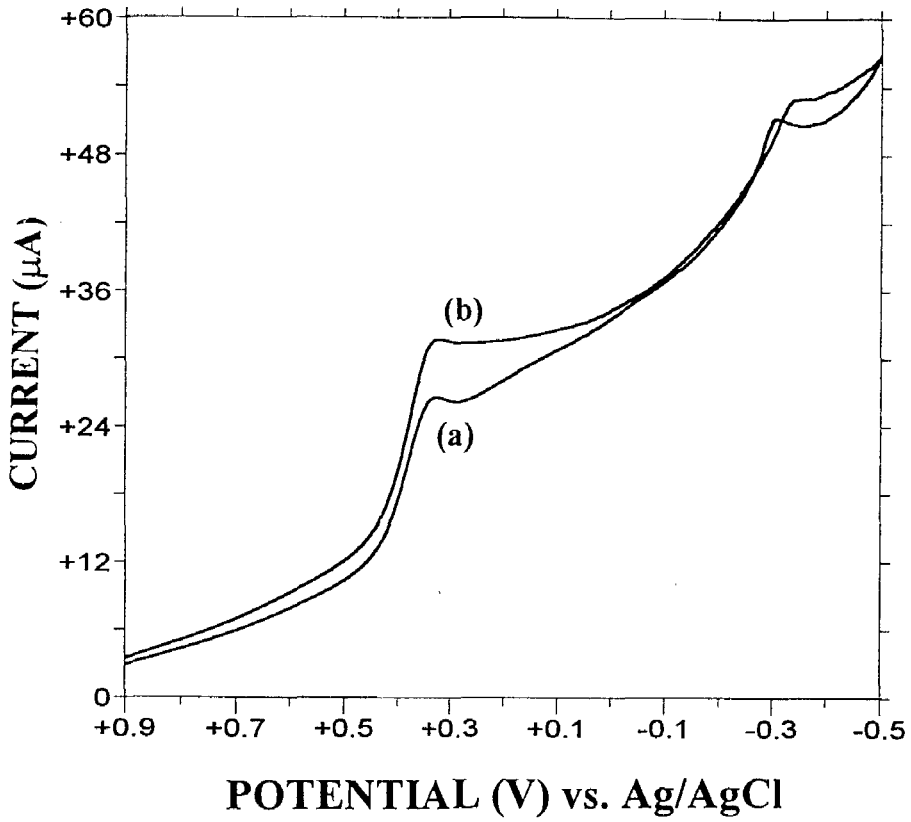

Figure 4.10: The AdCSV of gold(III) (6.00×10^{-5} mol dm^{-3}) and copper(II) (1.20×10^{-4} mol dm^{-3}) in the absence (a) and presence (b) of 0.24 μg l^{-1} of mannan. Scan rate = 100 mV s^{-1} Deposition potential = 1.05 V

The presence of copper(II) did, however, result in the formation of a new peak at -0.36 V; as illustrated in Figure 4.10(b). A weak reduction peak was observed for copper(II) alone in the presence of mannan near -0.27 V. The adsorptive cathodic stripping voltammogram of a solution containing gold(III) and copper(II), in the absence of mannan, is shown in Figure 4.10(a). The reduction peak at -0.36 V becomes sharper and more enhanced in the presence of gold(III) and the currents for the peak increase with an increase in the concentration of gold ions.

The addition of mannan, Figure 4.10(b), resulted in a larger enhancement in the observed peak associated with the reduction of the gold(III) complex than for the copper(II) complex, showing that the gold(III)-mannan complex formation is preferred over the copper(II)-mannan complex; even when the copper(II) is in a ten-fold excess to the gold(III) in solution.

The addition of increasing concentrations of copper(II) to the system, resulted in the peak at -0.36 V increasing very insignificantly in comparison to the amount of copper(II) added. In fact, addition of large concentrations of copper(II) ($> 10^{-3}$ mol dm^{-3}) resulted only in the broadening of the peak and not in an increase in current. The observation that the copper(II)-mannan complex, with a reduction potential at -0.36 V, was sensitive to the addition of gold(III), suggests the formation of inter-metallic compounds. The inter-metallic compound is thought to involve copper and gold ions and this compound is likely to have complexed with the mannan.

In the presence of both gold(III) and copper(II), the shift of 0.04 V in the potential of the peak associated with the reduction of gold(III), was smaller than the shift of -0.06 V observed for the reduction of copper(II) on addition of mannan. The observation of relevant peak shift suggests that the copper(II) may form a slightly more stable complex with mannan than the gold(III).

In spite of the proposed suggestion that copper(II) may form a slightly more stable complex with mannan than the gold(III),[84] the binding potential for gold(III) has been observed to be favoured over copper(II); when the copper(II) is in a ten-fold excess of the gold(III). Chemical modification of the *S. cerevisiae* cell wall has indicated the three chief functional groups involved in binding copper(II): namely the carboxyl, amino and hydroxyl groups.[84] However, the hydroxyl functional groups of *S. cerevisiae* have demonstrated a low binding affinity for copper(II) and since the majority of the binding sites on mannan contain hydroxyl functional groups, copper(II) would not be favoured.[84]

Figure 4.11 illustrates the behaviour of the stripping currents towards the gold(III) concentration for the reduction of the gold(III), with a constant ten-fold excess of copper(II), in the presence and absence of mannan.

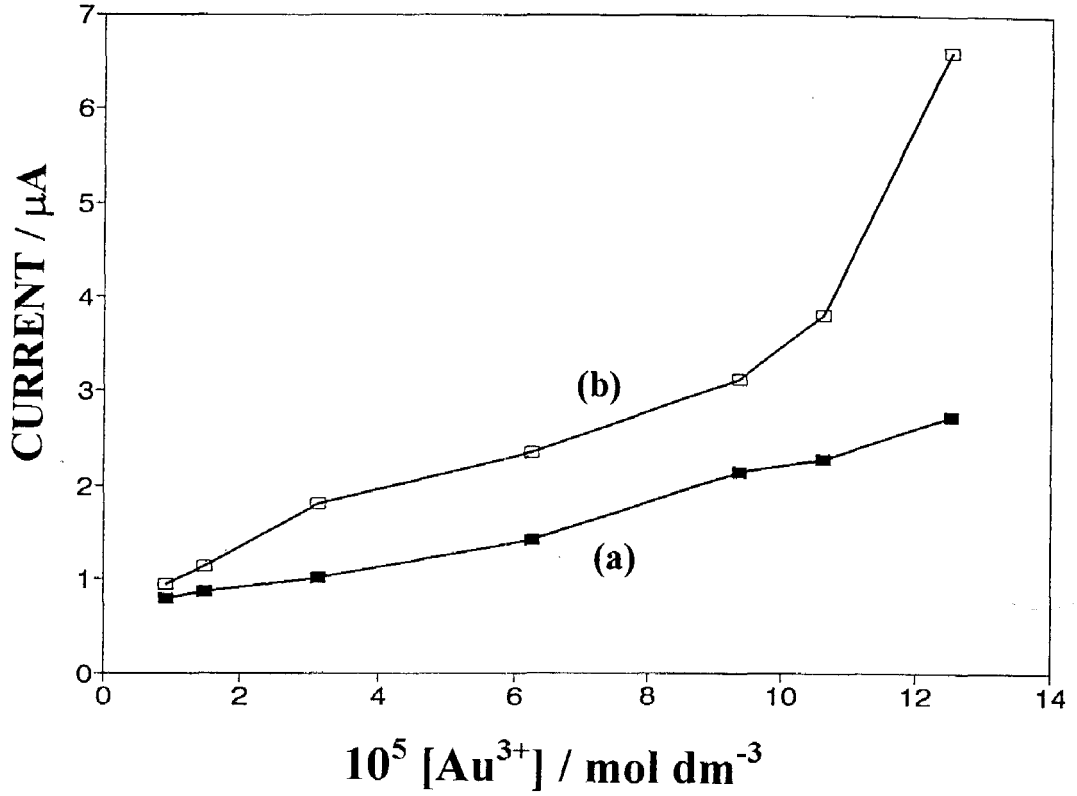

Figure 4.11: Concentration versus stripping current obtained for gold(III) in the absence (a) and presence (b) of mannan (0.30 μg l^{-1}) at a constant copper(II) level of 1.25 × 10^{-4} mol dm^{-3}. Scan rate = 100 mV s^{-1} Deposition potential = 1.05 V

A linear variance of stripping current vs. concentration was observed for the concentrations ranging from 1.50 × 10^{-5} mol dm^{-3} to 9.38 × 10^{-5} mol dm^{-3} of gold(III). Higher gold(III) concentrations (~10^{-4} mol dm^{-3}) emphasised the preference of mannan for gold(III) over copper(II). However, lower concentrations of gold(III) were affected by the formation of the inter-metallic gold(III)-copper(II) complex, hampering the detection of gold(III), in the presence of mannan, by lowering the number of gold(III) available for detection. The slow nucleation problem of gold ions on the glassy carbon electrode (referred to in section 1.3.4)

may also contribute to the variation of the gold reduction peak observed at lower gold(III) concentrations.

In the study performed on the AdCSV of gold(III) in the presence of silver(I) no significant changes in the adsorptive cathodic stripping voltammetry peak of the gold(III)-mannan complex were observed for silver(I) concentrations ranging from 1.00×10^{-4} to 1.00×10^{-3} mol dm^{-3}. No new peaks, which could be associated with the formation of a silver(I)-mannan complex, were observed; suggesting that silver(I) does not compete with gold(III) for the co-ordination of mannan under the present experimental conditions. However, the pyrolytic graphite and the glassy carbon electrodes have been shown to be unsuitable in the detection of small amounts of silver(I) in solution and carbon paste electrodes have been prescribed.[85]

Silver biosorption is strongly influenced by the pH of the solution, since silver is regarded as a 'soft' metal and the binding of such metals is predominantly a covalent mechanism involving hydrogen ion displacement. At a pH of 2.3, the silver(I) uptake had decreased by 74 % compared with the uptake capacity at pH 6.5.[21] The pH of the experimental solution, for gold(III)-mannan complex formation, was in the pH range of 0 - 2 pH units, which may account for the lack of a silver(I)-mannan complex forming.

4.3 Yeast cell wall modified carbon paste electrodes:

4.3.1 The mannan modified carbon paste electrode:

Mannan, was investigated for its binding properties, with respect to gold(III), utilising a mannan modified carbon paste electrode method. Mannan, as a suitable modifying agent, is required to lower the detection limit and/or increase the selectivity towards the metal ion of interest. The adsorptive cathodic linear sweep potential scan voltammetry technique, in the

presence of a nitric acid (0.10 mol dm^{-3}) media, was employed on the mannan modified carbon paste electrode for gold(III) detection.

A single cathodic stripping peak, in the absence of a mannan, was obtained at 0.30 V for gold(III) (1.30×10^{-8} mol dm^{-3}) employing the unmodified carbon paste electrode. The addition of the mannan to the carbon paste electrode increased the observed stripping current 100-fold and the peak associated with gold(III) reduction, shifted to 0.22 V.

The comparison between the gold(III) reduction peaks, in the absence and presence of mannan, is illustrated in Figure 4.12 (a) and (b) respectively. In the presence of mannan, a detection limit of 9.00×10^{-9}mol dm^{-3} was obtained. Mannan, thus meets the requirements of a suitable ligand, admixed into a carbon paste electrode, for gold(III) detection. The standard deviation of the stripping peak currents was observed to be less than 3 %.

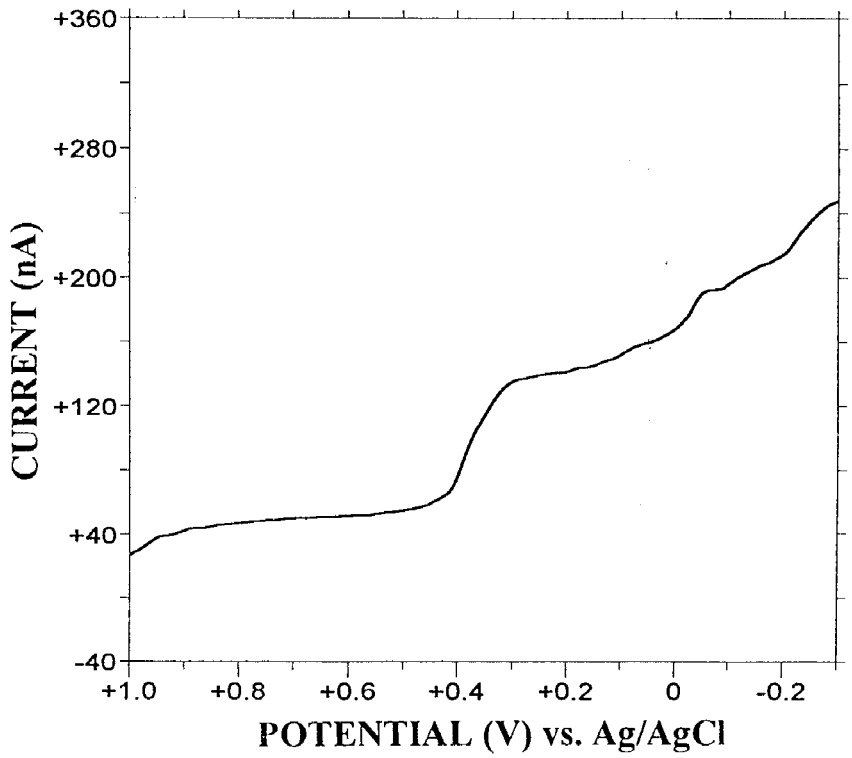

Figure 4.12(a): The gold(III) (1.30×10^{-8} mol dm^{-3}) reduction peak observed in the absence of mannan in the carbon paste electrode. Scan rate = 350 mV s^{-1}

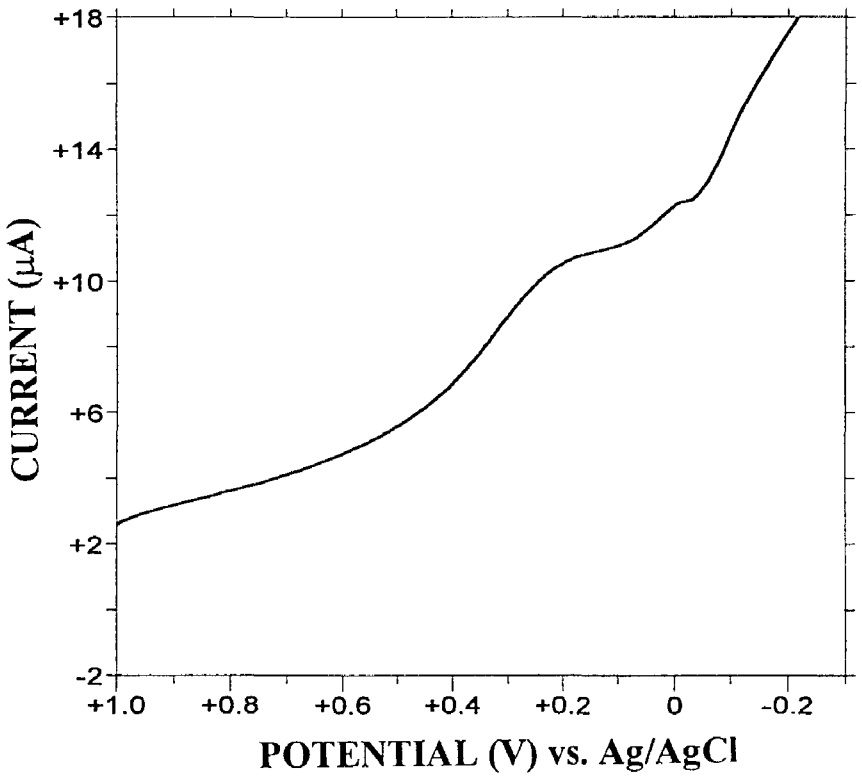

Figure 4.12(b): The gold(III) (1.30×10^{-8} mol dm^{-3}) reduction peak observed in the presence of mannan in the carbon paste electrode. Scan rate = 350 mV s^{-1}

The potential shift observed for the gold(III) reduction peak, in the presence and absence of mannan within the carbon paste electrode, is 0.08 V. The magnitude of the peak current shift obtained on the paste electrodes is larger than the shift obtained on the glassy carbon electrode for the AdCSV experiments in sections 4.1 and 4.2. The increased magnitude of the peak shift, associated with the gold(III) reduction, may be the result of the mannan being immobilised within the electrode. Studies have shown that the polyacrylamide immobilisation of *S. cerevisiae* allowed for the complete removal of certain metal ions such as copper(II), cobalt(II) and cadmium(II) from synthetic metal solutions.[86]

The optimum experimental conditions, attained in the AdCSV on a glassy carbon electrode, served as optimum conditions for the AdCSV modified carbon paste experiments. However, the scan rate and the deposition (accumulation) time were increased from 100 to 350 mV s^{-1} and from 40 seconds to 250 seconds respectively, since the concentration range

of the gold(III) was lowered to the order of 10^{-9} mol dm^{-3}. An added consideration in the preparation of the mannan MCPE, is the ratio of biomass/graphite/mineral oil. Figure 4.13 illustrates the optimum composition ratio of a mannan MCPE.

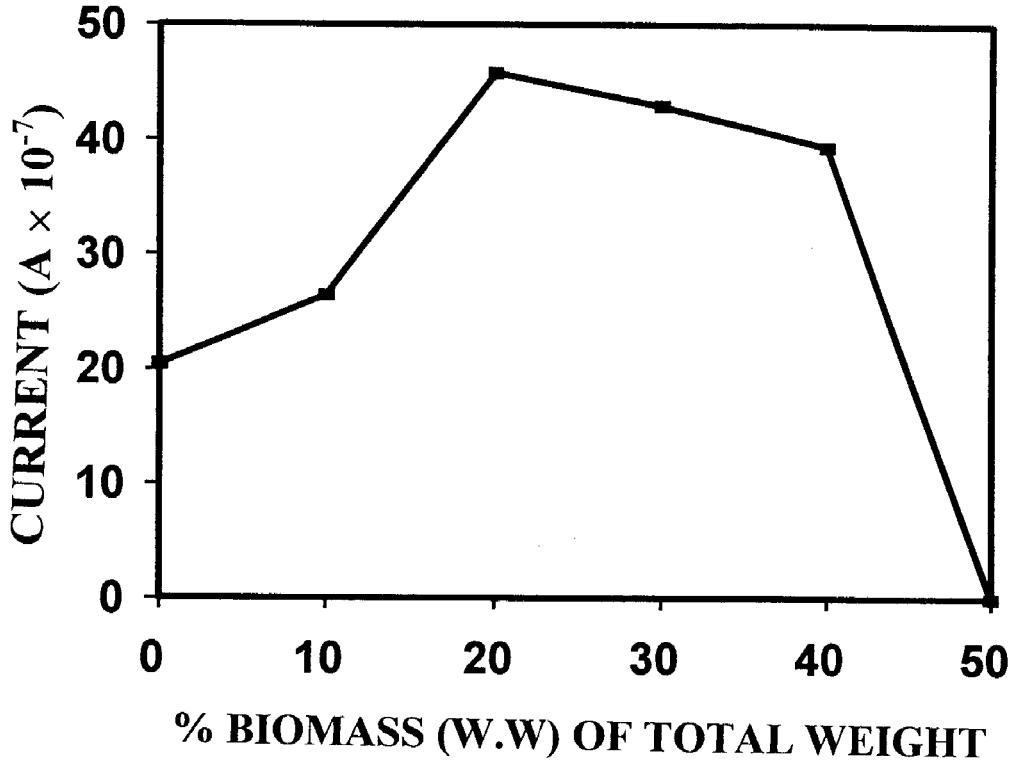

Figure 4.13: The optimum percentage biomass (w.w) per total weight, required for a modified carbon paste electrode (MCPE). Scan rate = 350 mV s^{-1}

The optimum composition ratio was found to contain a 20 % w.w of biomass per total composition weight. The 50 % biomass w.w per total weight electrode composition, did not render a stripping peak for the sample; since there was insufficient graphite powder/mineral oil (responsible for the conductivity of the electrode) present in the ratio.

The pH profile of the MCPE experiments is of interest; since the pH levels not only dictate the metal binding possibilities, but also give clues as to how the metal may bind to the biomass. The pH of the sample, used for the AdCSV of gold(III) on a mannan MCPE, was measured to be 0.7 pH units. At pH 1.0, a peak associated with the reduction of gold(III) was observed, but there were no visible peaks in this region around pH 2.0 and upwards. However, a small peak was noted several millivolts towards the negative region, but this peak faded rapidly as the pH levels increased.

The results, for the mannan pH profile, indicate that a highly acidic medium is necessary for the study of gold(III). Gold ion detection studies, via algal modified carbon paste electrodes, have observed a decrease in the gold(III) stripping peak upon increasing the pH between 2.00 and 5.00 pH units and no current responses are obtained at higher pH levels.[64]

Figure 4.14 illustrates the Osteryoung square wave adsorptive cathodic stripping voltammogram of gold(III) in the presence of mannan. Variations of the potential scan stripping technique, namely the Osteryoung and Barker square wave potential scan stripping forms, were applied to the MCPE experiments in an effort to lower the detection limits. The Barker square wave format resulted in a more complex voltammogram, however, the Osteryoung method enhanced the detection limit, for the reduction of gold(III) to gold(0) in the presence of 0.3 $\mu g\ l^{-1}$ mannan, to a favourable 1.70×10^{-11} mol dm^{-3}.

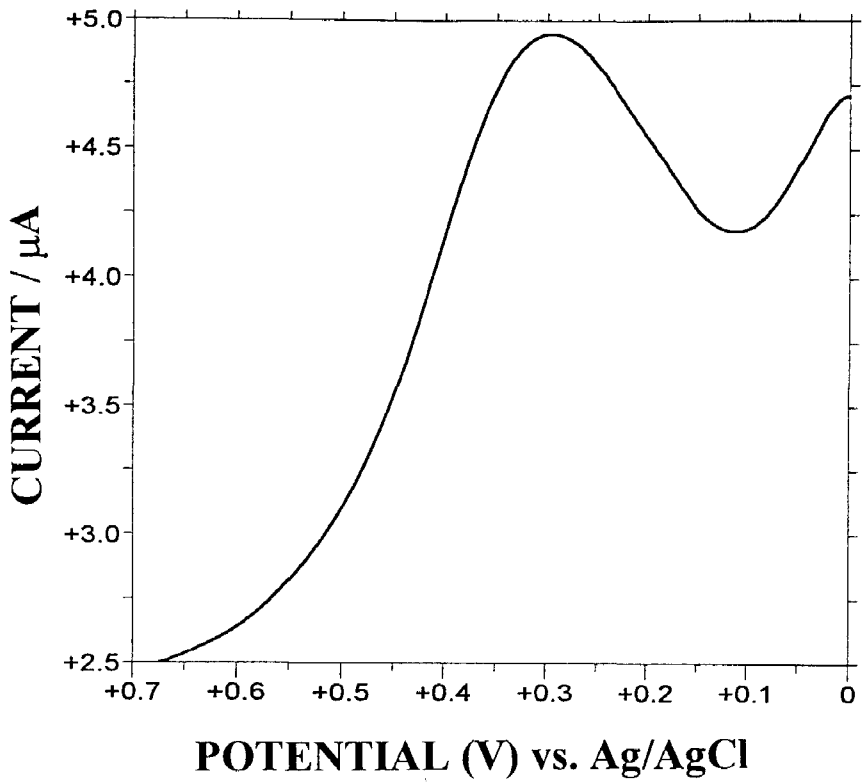

Figure 4.14: The Osteryoung square wave potential scan adsorptive cathodic stripping voltammogram of gold(III) (1.67×10^{-11} mol dm^{-3}) on a mannan modified carbon paste electrode. Scan rate = 350 mV s^{-1} Deposition potential = 1.05 V

4.3.2 The chitin and glucan modified carbon paste electrodes:

Chitin is a polymer of *N*-acetylglucosamine residues linked via glycosidic links, whilst glucan is a β(1,3) linked glucose polymer, with branched side chains at β(1,6) positions as mentioned in the introduction section and seen in Figure 1.2(b).[13] A small, broad peak, sensitive to changes in the gold ion concentration, was observed at 0.15 V for the chitin MPCE, and is illustrated in Figure 4.15.

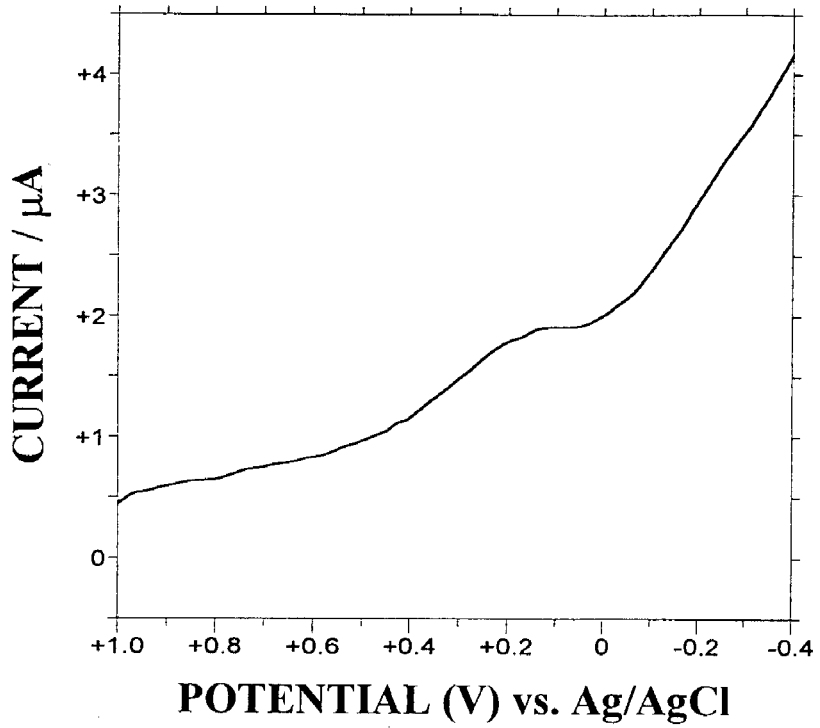

Figure 4.15: The gold(III) reduction on a chitin modified carbon paste electrode. Scan rate = 350 mV s^{-1} Deposition potential = 1.05 V

The optimum experimental conditions, for the mannan MCPE, were applied to both the glucan and chitin extracts. A detection limit, for gold(III) reduction, of 1.10×10^{-6}mol dm^{-3} was obtained for the chitin extract.

The glucan modified carbon paste electrode did not exhibit any peaks within the scanned potential window. Hydrogen ion release, a proposed pre-requisite for the binding of metal ions to hydroxyl containing complexes, will be affected under the acidic conditions specified. The glucan extract, with hydroxyl functional groups as possible binding sites, may thus be sensitive to the harsh acidic conditions. Metal-ligand interaction studies have also shown the dependency of the metal-ligand bond formation on the three-dimensional spacial arrangement of the ligands, which may be affected by changes in the environmental

conditions.[84] Chitosan, the remaining cell wall polysaccharide, could not be ground finely enough for the production of a MCPE, with the available grinding apparatus.

ADDITIONAL ANALYSIS TECHNIQUES FOR INVESTIGATING GOLD - BIOMASS INTERACTIONS:

5.1 The nuclear magnetic resonance (NMR) experiments:

The study of molecular structure, conformational changes, interaction of biological molecules with various substrates and certain types of kinetic investigations are the main uses of NMR in the biological field.[67] Polysaccharides which are composed of regular repeating units, such as most bacterial and yeast polysaccharides, lend themselves readily to NMR spectroscopy. A spectrum of such a compound is essentially that of a single repeating unit, regardless of the degree of polymerisation of the compound. However, increasing the molecular mass will result in line broadening, due to an increase in the viscosity of the polysaccharide sample.[69]

A typical one dimensional (1D) ^{1}H NMR spectrum of a micro-organism polysaccharide is fairly complex, due to the degree of overlap of the signals arising from the ring protons. The remaining region of the spectrum of a polysaccharide, which lacks methyl groups, is referred to as the anomeric region. Should a metal-ligand complex form between the polysaccharide and the metal, the signals relating to the hydrogen atoms near the complexation site will shift; usually downfield. This enables the site of complex formation to be pinpointed. In this study the greatest shift was observed in the anomeric signals, implying complexation near carbons 1 and 2 of the individual sugar units.

All NMR data collected was obtained at an experimentally optimised temperature of 30 °C, which allowed for the detection of any possible sample absorption peaks that may have occurred under the HOD (Hydrogen Oxygen Deuterium) peak at other operating temperatures. The initial NMR experiments were carried out on the yeast mannan extract in 99.99 % D_2O in an attempt to ascertain its structure; since to date, the NMR elucidation of

the mannan complex had not been performed. The assignment of the chemical shift values was carried out using the COSY spectrum; see Figure 5.1.

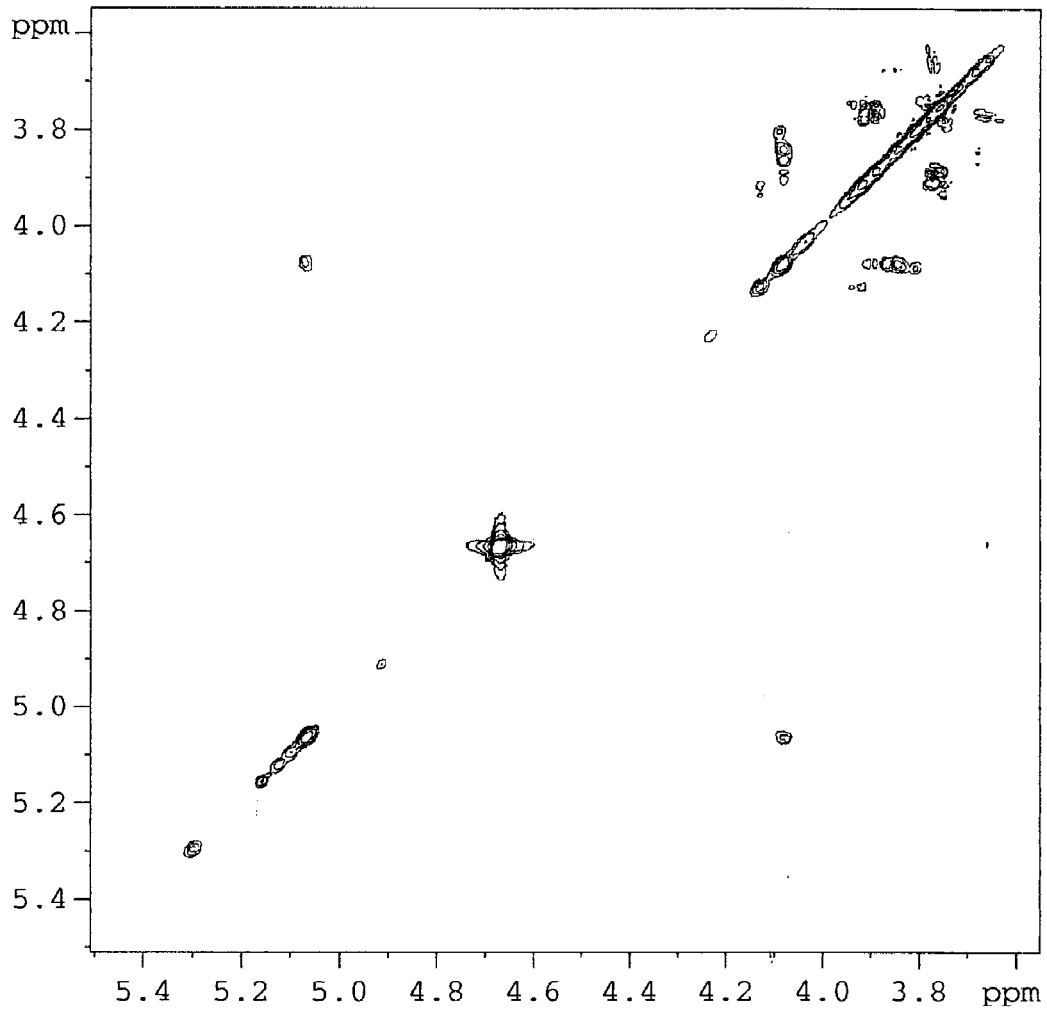

Figure 5.1: The COSY spectrum obtained for the mannan extract at 30 °C.

The main aim of the NMR study is to verify the proposed metal - biomass complex formation, observed in the adsorbed cathodic stripping voltammetry experiments, and to obtain information concerning the binding positions on the polysaccharide. The adsorbed

cathodic stripping voltammetry experiments were all performed under harsh acidic conditions and hence the affects of acidifying the sample solution were significant. No major changes were observed on the addition of acid to the mannan sample, in the absence of gold ions; as can be seen by comparing Figures 5.2 and 5.3.

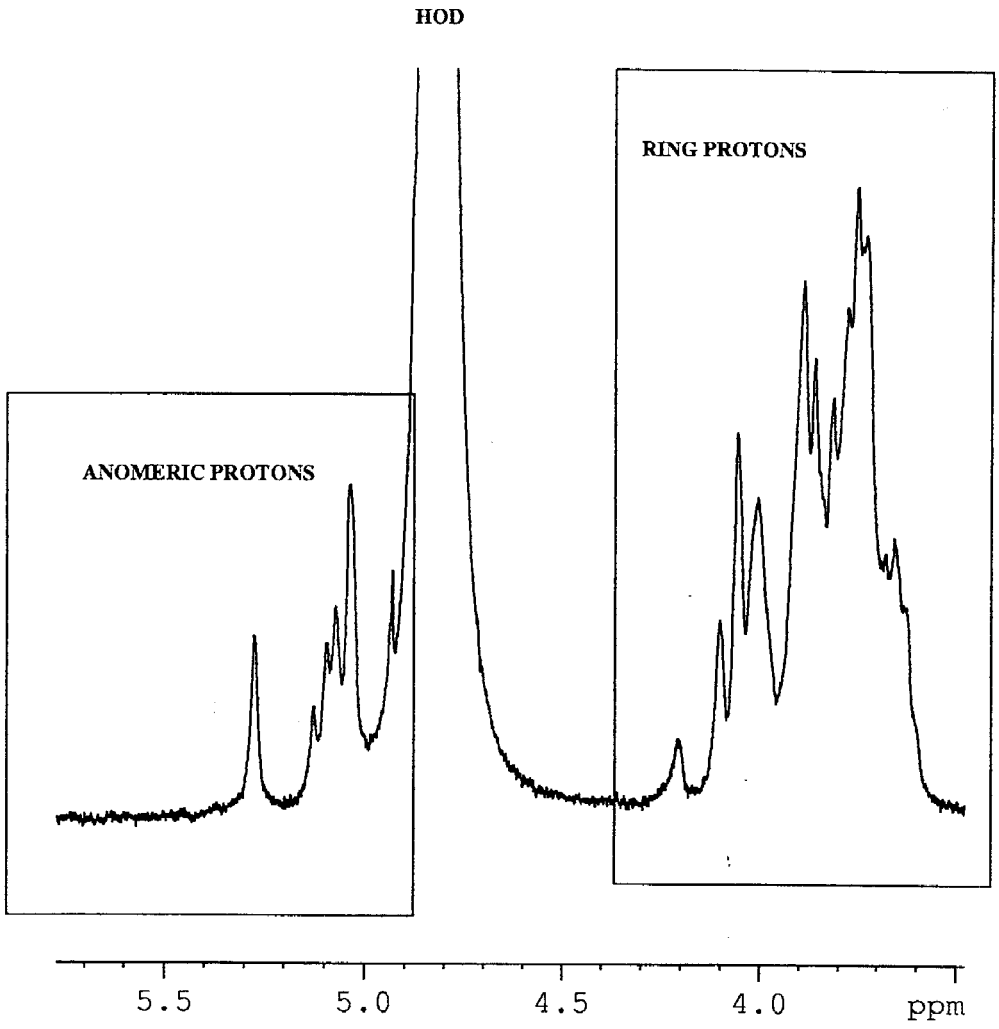

Figure 5.2: The 1D ^{1}H NMR spectrum obtained for mannan (1 mg ml^{-1}) under non-acidified conditions at a temperature of 30 °C.

The chemical shift values and coupling constants provide information concerning the anomeric configuration and permit the identification of the basic configuration of the

monosaccharide. NMR experiments on residues containing the *manno* configuration have been attempted and the configuration has been noted to be problematic for structure elucidation experiments.

The problem occurs as a result of the signal for the anomeric proton resonating close to 5.00 ppm and the coupling constants for the α- and β-linked sugars being small. However, in such cases the configuration may be assigned from the $J_{C\text{-}1,H\text{-}1}$ values or the Nuclear Overhauser Enhancement data.[69]

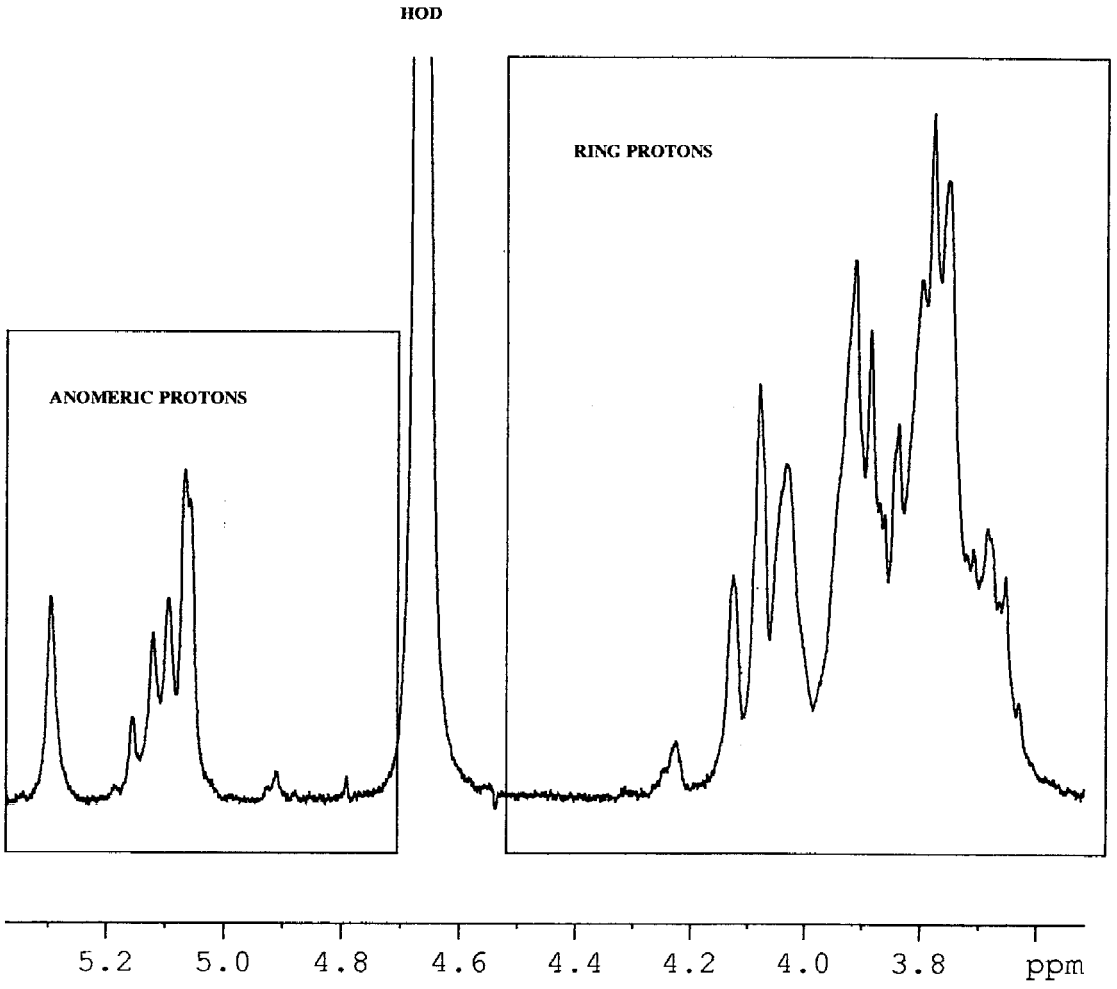

Figure 5.3: The 1D ^{1}H NMR spectrum obtained for mannan (1 mg ml^{-1}) under acidified conditions at a temperature of 30 °C.

Figure 5.4 depicts the 1D ^{1}H NMR spectrum obtained for mannan in the presence of 100 µl of gold(III) and under nitric acid acidified conditions. The optimum NMR experimental parameters, obtained with the mannan investigation (Figures 5.2 and 5.3), were maintained for the gold-mannan complex formation NMR studies.

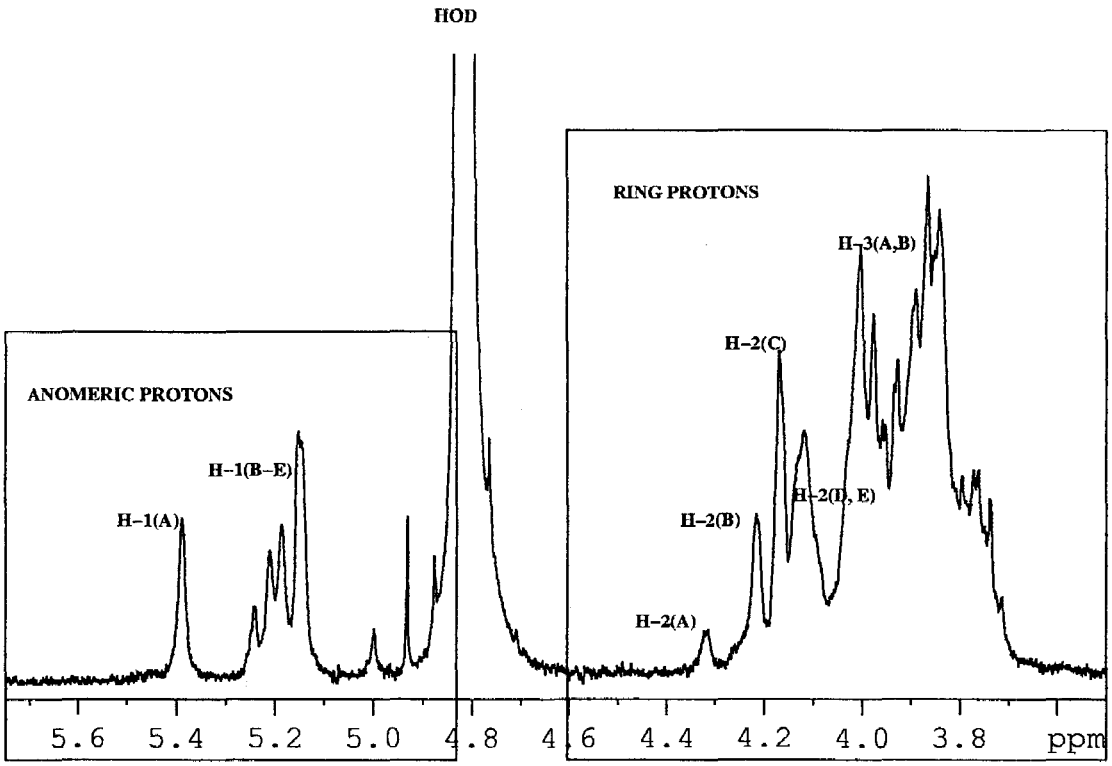

Figure 5.4: The 1D ^{1}H NMR spectrum obtained for mannan (1 mg ml^{-1}) under acidified conditions in the presence of 100 µl of gold(III) (10^{-3} mol dm^{-3}) at 30 °C.

The NMR data obtained in the absence (Figure 5.2) and presence of gold(III) (Figure 5.4) has been summarised in Table 5.1 and 5.2 respectively. The polysaccharide monomer units were randomly assigned labels from (A) to (E) in order of their decreasing chemical shift values. In the cases where no values have been entered in Table 5.1, the chemical shift value could not be assigned to that monomers specific proton as a result of chemical shift values overlapping.

Table 5.1: Summary of the NMR data in the absence of gold(III):

Mannan in absence of gold(III)	Monomer unit (A) (ppm)	Monomer unit (B) (ppm)	Monomer unit (C) (ppm)	Monomer unit (D) (ppm)	Monomer unit (E) (ppm)
H-1	5.275	5.127	5.097	5.072	5.036
H-2	4.205	4.108	4.007	-	4.058
H-3	3.891	3.864	-	-	-

Table 5.2: Summary of the NMR data in the presence of gold(III):

Mannan in presence of gold(III)	Monomer unit (A) (ppm)	Monomer unit (B) (ppm)	Monomer unit (C) (ppm)	Monomer unit (D) (ppm)	Monomer unit (E) (ppm)
H-1	5.389	5.241	5.210	5.186	5.151
H-2	4.314	4.217	4.119	-	4.170
H-3	3.976	3.958	-	-	-

The comparison between Tables 5.1 and 5.2 indicate a strong downfield shift in the various chemical shift values observed. In particular the absorption peaks belonging to H-1 and H-2 protons showed the greatest changes; this implies that the H-1 (Δ 0.11 ppm) and H-2 (Δ 0.10 ppm) protons may be involved in the gold(III)-mannan complex formation. Chemical shift differences of 0.1 ppm and greater were observed for these protons. The downfield shift is indicative of deshielding of the protons involved.

Chemical shift differences in the order of 0.1 ppm are suggestive of co-ordination of the complex by the co-ordination agent.[68] A further indication of a possible metal-ligand complex formation occurring, is the enhancement of the spectrum resolution on addition of the metal ions and this is illustrated when comparing Figure 5.3 in the absence of gold(III) with Figure 5.4 in the presence of gold(III).

5.2 The infrared spectroscopy study:

The stretch vibrations of the oxygen-hydrogen and the carbon-oxygen bonds are responsible for the characteristic absorptions in the polysaccharide infrared spectroscopy experiments. The strong absorption peaks in the region of 3335 cm^{-1} are due to of the hydroxyl groups present in the sugar and alcohol molecules. The carbon-oxygen stretch appears between 1250 and 1000 cm^{-1}; depending on the degree of substitution of the carbon atom. The polysaccharide spectra characteristically exhibit broad bands in comparison to the spectra observed for the simple alcohol molecules. The polysaccharide spectra do not display the aldehyde carbonyl absorption peaks, due to their cyclic hemiacetal structure.[87]

Figure 5.5 illustrates the infrared spectrum of mannan obtained from a KBr disc. The spectrum was run from 4000 cm^{-1} to 1000 cm^{-1}. A large, broad, unresolved peak corresponding to the hydroxyl absorption was observed in the 3200 to 3600 cm^{-1} range. At approximately 3000 cm^{-1} a sharp, clearly defined peak is noted. Peaks corresponding to the carbon-hydrogen stretchings are observed around 1600 and 1400 cm^{-1}. The large absorption peaks observed in the 1000 cm^{-1} are the result of the carbon-oxygen vibrations.

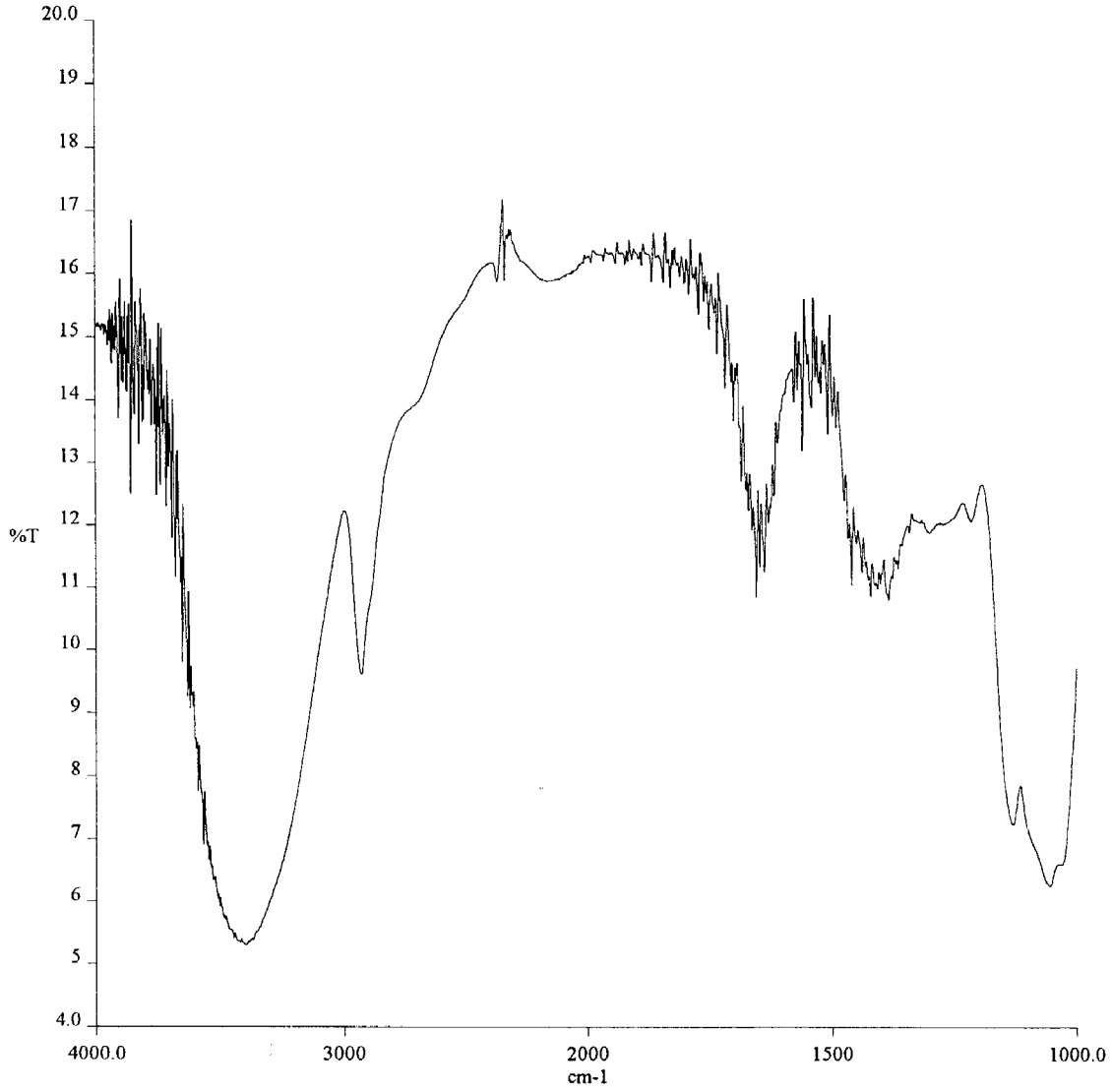

Figure 5.5: The infrared KBr disc spectrum obtained for the mannan extract.

The effect of the addition of gold(III) to a mannan sample on the IR spectra is observed in Figure 5.6. The mannan extract and the proposed gold(III)-mannan complex are highly water soluble, hence the sample from the NMR study, in D_2O and under acidic conditions, was evaporated on a high vacuum rotary evapourator to a solid product. The presence of D_2O affects the spectrum slightly; peaks observed between 2000 and 2400 cm^{-1} are the result of D_2O present. However, the region of interest, ~3000 to 3600 cm^{-1}, on addition of gold(III) to the mannan, still exhibited changes associated with the possible co-ordination at the hydroxyl sites.

The spectrum obtained in the presence of metal ions was more highly resolved, especially in the 3000 cm^{-1} region. Four major absorption peaks were observed in this region, with the latter two peaks having been shifted from the expected hydroxyl region. The third absorption peak is indicative of co-ordination at the hydroxyl site.

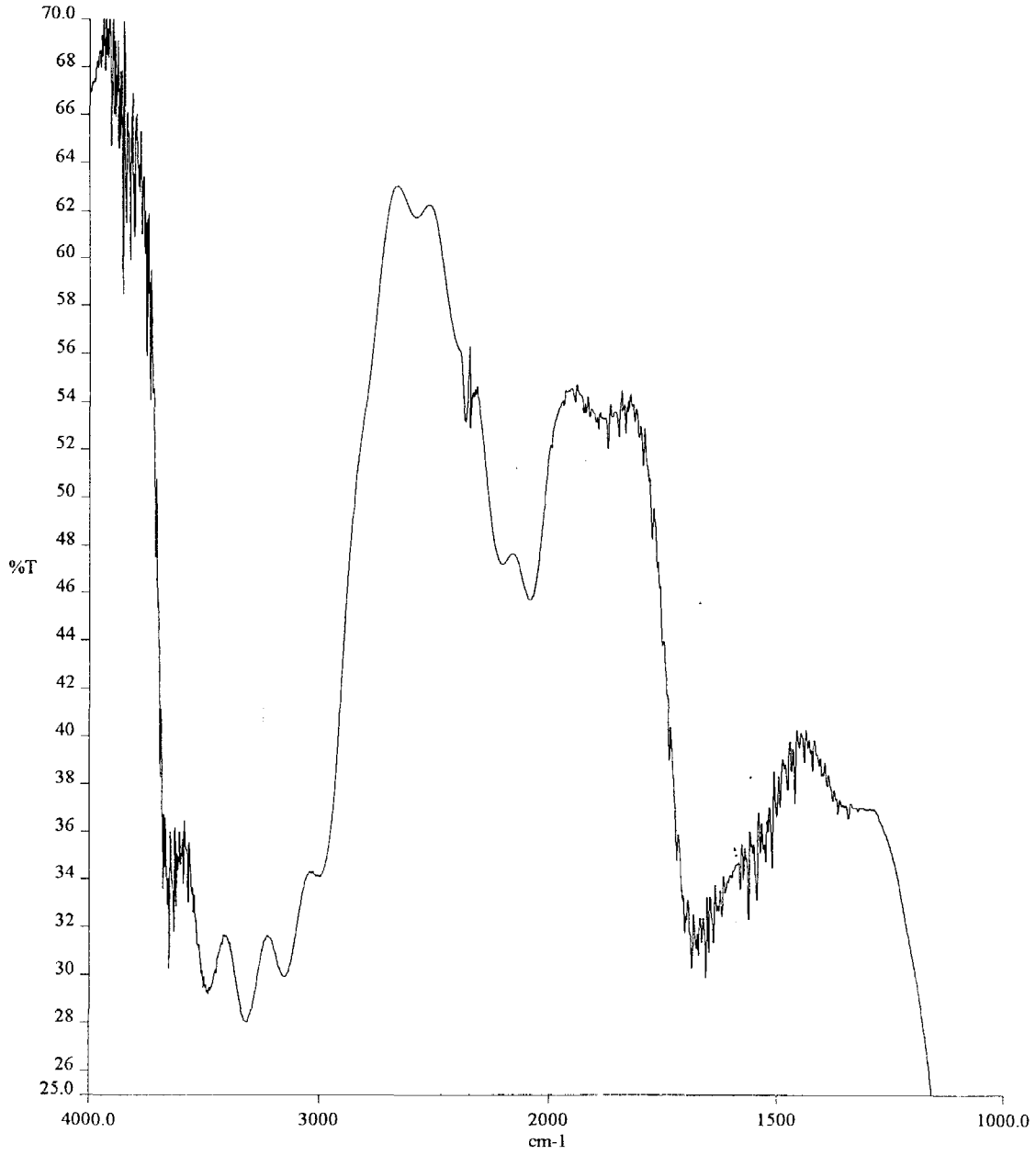

Figure 5.6: The infrared spectrum obtained for the gold(III)-mannan complex.

5.3 Computer modelling and energy minimisation techniques:

The computer modelling technique employed high quality computer graphics as an aid in visualising the molecules, molecular processes and intermolecular interactions. A common use of the molecular visualisation technique is the interaction of metals with macromolecules.

The metal-macromolecule interactions are inevitably complex and molecular modelling and visualisation can be of enormous assistance in understanding the nature of the interactions and the factors that mediate them. However, as a result of the complexity of the metal-macromolecule system, the model generated is but one of the possible representations of the interaction and the model corresponds to one of the many possible energy minima on a complex potential energy surface.[66]

The Newton-Raphson energy minimisation technique was employed for all the models generated. The technique converges very efficiently for molecules close to the optimum structure as a result of the potential energy surfaces being close to the harmonic near the energy minimum.

Figure 5.7 is a graphic representation of a section (for simplification purposes of the graphic model, about five repeating mannose units including a side chain section were represented as the molecule), of the minimised mannan extract. On addition of gold(III) to the system (Figure 5.8(a) and (b)), changes in the position of the biological molecules were observed and a square-planar geometry was found to exist. The experimentally found geometry is in agreement with the trend observed for gold(III) according to the literature.[38]

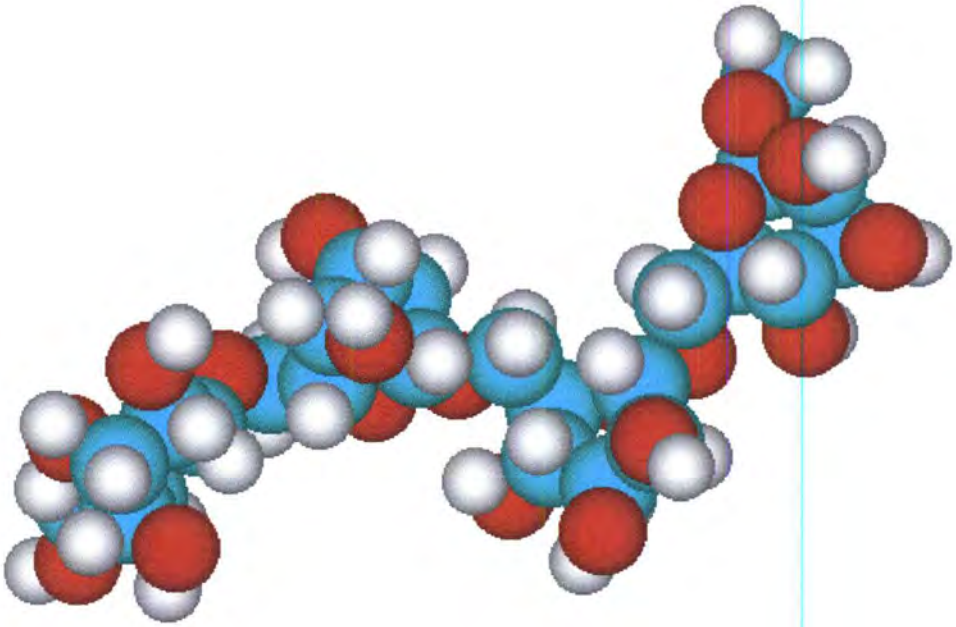

Figure 5.7 The Newton-Raphson energy minimised structure for a section of the mannan biological macromolecule.

Colour Key	Colour	Element
	Blue	Carbon
	Red	Oxygen
	White	Hydrogen

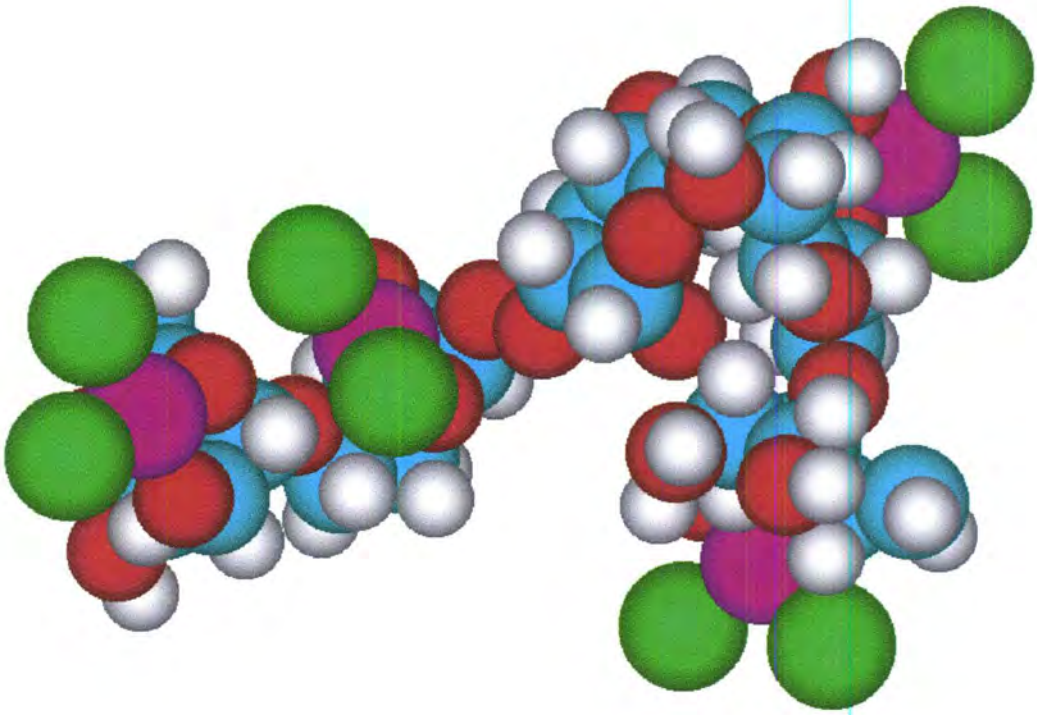

Figure 5.8(a): The space-filling model of the proposed gold(III)-mannan complex, indicating the favoured square-planar geometry adopted by the gold.

Colour Key	Colour	Element
	Violet	Gold(III)
	Green	Chlorine
	Blue	Carbon
	Red	Oxygen
	White	Hydrogen

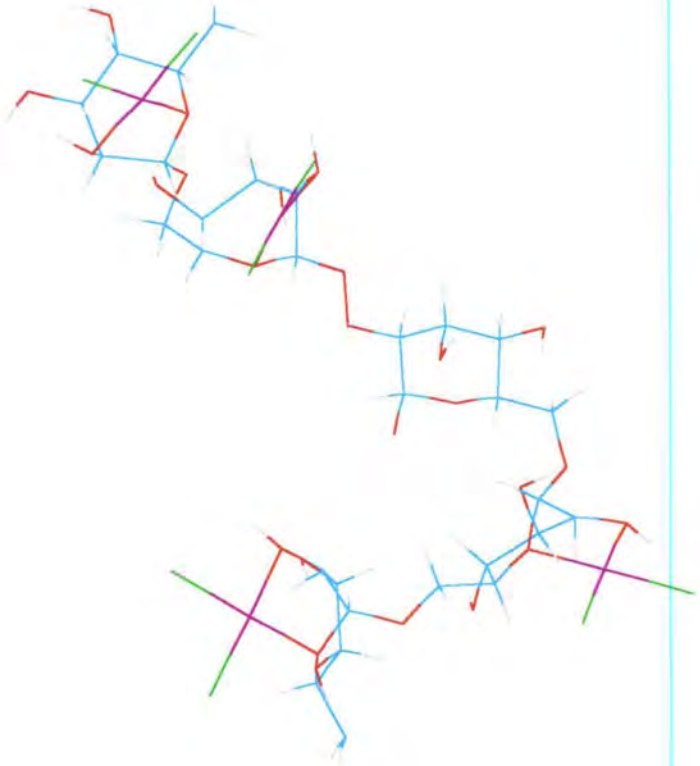

Figure 5.8(b): The stick model illustrating a section of the proposed gold(III)-mannan complex.

THE CONCLUSION:

The electrochemical stripping voltammetry has proven its usefulness in the determination of gold ions in solution. The optimum parameters for the detection of gold(III) varies largely with the type of electrochemical method employed, the choice of working electrode surface, the electrolyte and the presence of any interfering ions in solution.

The presence of mercury(II) may serve as a co-depositing material or for amalgamation formation, which will be stripped off before any gold peaks are observed, and thus over-come the poor nucleation problem; as observed in the successful anodic stripping voltammetry gold(III) detection experiments. However, in the conventional anodic stripping voltammetry experiments, employing the use of a mercury film, fails for the detection of gold ions, since the oxidation potential of mercury is considerably less positive than that of gold.

The mercury co-deposition and/or film formation method is not suitable for the detection of gold ions in a complex matrix; such as the mine effluent sample. Gold(III) detection requires the use of harsh acidic conditions and a type of *aqua regia* was utilised successfully in the application of the stripping voltammetry technique to the mine water sample.

In this research the mannan extract has proven to be the probable cell wall extract responsible for the bioremediation of gold(III) from solution. However, the metal-ligand complex formation has been shown to be dependent on the three-dimensional structure of the ligand and hence the entire structure of the cell wall, including the remaining cell wall extracts and the proteins associated with them, should be taken into account in the metal binding process.

The *S. cerevisiae* mannan extract has demonstrated an affinity for both gold(III) and copper(II); as seen in the linear sweep potential scan adsorptive cathodic stripping

voltammetry experiments. The copper(II) may compete with gold(III) for the co-ordination sites on mannan; resulting in significant interferences for the accumulation of gold by yeast mannan extract. However, the mannan extract was found to be more sensitive to gold(III) than to copper(II) and the detection limit (9×10^{-9} mol dm^{-3}) for the gold(III) reduction compares favourably with the literature.

Despite the fact that high sensitivity levels are an important requirement from an analytical view point, the sensitivity levels must be accompanied by high selectivity levels for an optimum detection limit to be achieved. The observation that silver(I) does not compete with gold(III) for mannan co-ordination sites, under the chosen experimental conditions, is significant; since silver(I) is known to cause serious interferences for the determination of gold(III) in solution.

The NMR, IR and computer modelling data collaborate with the electrochemical experiments and the NMR data was useful in suggesting the binding position on the mannan extract for the gold(III). The gold ion has been shown to adopt the conventional square-planar geometry when bound to the mannan, via the H-1 and H-2 proton sites.

The development of ligands that are specific for gold ions is important in the analysis and recovery of gold. The study of the gold detection and the interaction of the gold ions with the *S. cerevisiae* cell wall extracts, represents a significant step towards better understanding the nature of gold chemistry, the electrochemistry of the metal and the accumulation of gold via biosorption.

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